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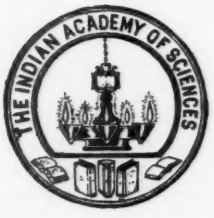

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A NOTE ON POISSON DISTRIBUTION

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(Communicated by Prof. K. B. Madhava, F.A.SC.)

1. *Introduction*

Poisson distribution like the binomial, contains only one parameter and is of very wide application in statistical work. The usual estimate for the parameter is the sample arithmetic mean obtained by the method of maximum likelihood. The method of least squares yields in this case an estimate that cannot be expressed as a convenient function of the observed values. A slight modification in the method of approach to the problem of estimation allows a direct application of the least square principle and gives an estimate which is elegant though not as simple as the maximum likelihood estimate. A discussion on the estimate so obtained is given in this paper.

2. *Estimate for* m

Let the population frequencies be denoted by

$$Y_k = \frac{N \cdot m^k e^{-m}}{\Gamma(k+1)}, \qquad (k = 0, 1, 2, \cdots) \qquad (1)$$

and the observed frequencies by

$$y_k \qquad (k = 0, 1, 2, \cdots) \qquad (2)$$

Also

$$N = y_0 + y_1 + y_2 + \cdots + y_n.$$

We have in the population

$$Y_{k+1} = \frac{m}{k+1} \cdot Y_k \qquad (3)$$

Since the relation (3) exists in the population, we shall assume that in a sample also an approximately similar relationship holds good. Hence

$$y_{k+1} = \frac{m}{(k+1)} \cdot y_k + \epsilon_k \qquad (k = 0, 1, \cdots n-1) \qquad (4)$$

where the ϵ's are small. To estimate m we minimise

$$\sum_0^{n-1} \epsilon_k^2 = \sum_0^{n-1} \left[y_{k+1} - \frac{m}{k+1} \cdot y_k \right]^2.$$

By differentiation, we get the required estimate

$$m_2 = \frac{\sum_0^{n-1} [y_k \cdot y_{k+1}/(k+1)]}{\sum_0^{n-1} [y_k^2/(k+1)^2]} \quad * \qquad (5)$$

It may be interesting to note that in the case of the binomial distribution

$$Y_{k+1} = \frac{n-k}{k+1} \cdot \frac{p}{1-p} Y_k.$$

Using the method adopted above the estimate for p is

$$\Sigma \frac{n-k}{k+1} \cdot y_k \cdot y_{k+1} \Big/ \left[\Sigma \left(\frac{n-k}{k+1} y_k \right)^2 + \Sigma \frac{n-k}{k+1} \cdot y_k \cdot y_{k+1} \right].$$

The statistics m_2 satisfies the criterion of consistence, for the population value of m_2 is given by

$$\frac{N^2 \cdot e^{-2m} \cdot \sum_0^{n-1} \frac{m^{2k+1}}{\Gamma(k+2) \cdot \Gamma(k+2)}}{N^2 \cdot e^{-2m} \cdot \sum_0^{n-1} \frac{m^{2k}}{\Gamma(k+2) \cdot \Gamma(k+2)}} = m$$

Though the statistic is a consistent one, it is clear that it does not satisfy the criterion of sufficiency.

3. *Efficiency of* m_2

To determine the efficiency of the estimate m_2, we proceed to calculate the standard error of m_2 in large samples.

We have

$$m_2 = u/v \text{ where} \qquad (6)$$

$$\left.\begin{aligned} u &= \sum_0^\infty y_k \cdot y_{k+1}/(k+1); \qquad v = \sum_0^\infty y_k^2/(k+1)^2 \\ \text{Also let} \qquad & \qquad (7) \\ U &= \sum_0^\infty Y_k \cdot Y_{k+1}/(k+1); \quad V = \sum_0^\infty Y_k^2/(k+1)^2 \end{aligned}\right\}$$

In repeated samples, let us denote the small variation in the class frequency y_k by δy_k $(k = 0, 1, 2, \cdots)$ and the corresponding variations in u, v, m_2, by δu, δv and δm_2 respectively. Hence

$$\left.\begin{aligned} y_k &= Y_k + \delta y_k \ (k = 0, 1, \cdots \infty) \qquad \dagger \\ u &= U + \delta u, \ v = V + \delta v, \ m_2 = m + \delta m_2. \end{aligned}\right\} \qquad (8)$$

---

* We shall assume that m_1 denotes the max. likelihood estimate, *viz.*,

$$m_1 = (y_0 + y_1 + \cdots + y_n)/n$$

† We assume $y_k = 0$ $(k = n+1, n+2, \cdots)$.

From (6) and (8) we have

$$V \cdot \delta m_2 = \delta u - m_2 \cdot \delta v. \tag{9}$$

Further,

$$\delta u = \sum_0^\infty [Y_k \cdot \delta y_{k+1} + Y_{k+1} \cdot \delta y_k] \div (k+1) \tag{10}$$

$$\delta v = \sum_0^\infty [2Y_k \cdot \delta y_k] \div (k+1)^2. \tag{11}$$

Using (10), (11) and (3) in (9), we have,

$$V \cdot \delta m_2 = \sum_1^\infty \left[\frac{Y_{k-1}}{k} - \frac{Y_{k+1}}{k+1}\right] \cdot \delta y_k - Y_1 \cdot \delta y_0 \tag{12}$$

Let

$$P_k = \frac{Y_{k-1}}{k} - \frac{Y_{k+1}}{k+1} \tag{13}$$

Equation (12) can now be written as

$$V \cdot \delta m_2 = \sum_1^\infty P_k \cdot \delta y_k - Y_1 \cdot -\delta y_0. \tag{14}$$

Squaring (14) and taking expectations,

$$V^2 \cdot E(\delta m_2^2) = \sum_1^\infty P_k^2 \cdot E(\delta y_k^2) + Y_1^2 \cdot E(\delta y_0^2) - 2Y_1 \cdot \sum_1^\infty P_k \cdot E(\delta y_0 \cdot \delta y_k) + \sum_{k \neq j}^\infty \sum^\infty P_k \cdot P_j \cdot E(\delta y_k \cdot \delta y_j) \tag{15}$$

Since

$$E(\delta y_k^2) = Y_k \cdot (1 - Y_k/N) \text{ and}$$

$$E(\delta y_k \cdot \delta y_j) = -Y_k \cdot Y_j/N \qquad (k \neq j)$$

Equation (15) becomes,

$$V^2 \cdot \sigma_2^2 = \sum_1^\infty P_k^2 \cdot Y_k + Y_1^2 \cdot Y_0 - \frac{1}{N}\left[\sum_1^\infty P_k^2 \cdot Y_k^2 + \sum_{k \neq j}^\infty \sum^\infty P_k \cdot P_j \cdot Y_k Y_j - 2Y_0 \cdot Y_1 \sum_1^\infty P_k Y_k + Y_0^2 \cdot Y_1^2\right]$$

$$= \sum_1^\infty P_k^2 \cdot Y_k + Y_1^2 \cdot Y_0. \tag{16}$$

Substituting the value of P_k, we get after reduction,

$$\sigma_2^2 = \frac{e^m}{N} \frac{\sum_1^\infty \frac{m^{3k-1}}{k\,(k\,!)^3} - 2\sum_1^\infty \frac{m^{3k}}{k\,!\,(\lfloor k+1)^2} + \sum_1^\infty \frac{m^{3k-2}}{(k\,!)^3}}{\left[\Sigma \frac{m^{2k}}{(\lfloor k+1)^2}\right]} \tag{17}$$

---

* We assume that σ_1 and σ_2 are the standard errors of m_1 and m_2 respectively.

It has not been possible to obtain σ_2 in terms of elementary functions. The denominator can be expressed in terms of known series, it being equal to

$$[I_0(2m) - 1]^2 m^4$$

where $I_0(2m)$ is the Bessel Function of order zero with a purely imaginary argument. However the other series are rapidly converging and for particular values of m the sum may be calculated to any degree of accuracy.

The standard error of m_2, makes it possible to calculate the efficiency E of m_2, the standard error of the maximum likelihood estimate being equal to m/N

$$E = \sigma_1^2/\sigma_2^2. \tag{18}$$

For small values of m Table I below gives E. For large values of m the numerical calculation is heavy and hence we proceed to find an asymptotic formula for E.

The asymptotic expansions for the several series in equation (17) are as follows:—

$$I_0(2m) \sim \frac{e^{2m}\,[1 + 0\,(1/m)]}{2 \cdot \sqrt{m\pi}}\dagger \tag{19}$$

$$\sum_1^\infty \frac{m^{2k-1}}{k \cdot (k!)^3} \sim e^{2m}\left[1 + \frac{7}{9m} + 0\,(1/m^2)\right] \div (2\pi \cdot \sqrt{3} \cdot m^3) \tag{20}$$

$$\sum_1^\infty \frac{m^{3k}}{\underline{k} \cdot (\underline{k+1})^2} \sim \frac{e^{3m}\,[1 - 2/9m + 0\,(1/m^2)}{2\pi\sqrt{3}m^3} \tag{21}$$

$$\sum_1^\infty \frac{m^{2k-2}}{(k!)^2} \sim \frac{e^{3m}\,[1 + 1/9m + 0\,(1/m^2)}{2\pi \cdot m^3 \cdot \sqrt{3}} \tag{22}$$

Using these expansions, we get

$$E = 3 \cdot \sqrt{3}/8 = 0{\cdot}6495.$$

TABLE I

Efficiency of m_2

m ..	0	·5	1	1·5	2	3	4	5	10	∞
E ..	1	·4929	·3617	·3182	·3542	·4481	·5215	·5583	·5910	·6495

4. *Numerical Illustration*

In the example given below both m_1 and m_2 are calculated and the corresponding values of the frequencies tabled and compared.‡

† See *Asymptotic Developments of Functions defined by Series*, Walter B. Ford, p. 85. For (20), (21) and (22) see appendix.

‡ The example is taken from *The Theory of Probability*, Jeffries, p. 59.

TABLE II

The Frequency of Nuclei of Condensation in Aitken Dust Counter

Number	0	1	2	3	4	5	6	7	8
Observed Frequency	23	56	88	95	73	40	17	5	3
Calculated Frequency—									
1. m_1 (=2·93)	25	65	88	82	61	38	21	10	4
2. m_2 (=2·99)	20	60	90	90	67	40	20	9	3
Deviations—									
1. Based on m_1	−2	−9	..	13	12	2	−4	−5	−1
2. Based on m_2	3	−4	−2	5	6	0	−3	−4	0

Root mean square when m_1 is the estimate = 7·0

do. m_2 do. = 3·6.

5. *Conclusion*

The object of this paper has been to get an estimate for the parameter in the Poisson distribution, based on the method of least squares. The statistic obtained is found to satisfy the condition of consistence; it is, however, not a sufficient statistic. Its standard error has been found and its efficiency determined, and an asymptotic value for the latter obtained.

In conclusion, the author wishes to thank Dr. U. Sivaraman Nair for his kind help in the preparation of this paper. The author is also grateful to the University of Travancore for allowing him to carry on this work.

6. *Mathematical Appendix*

In what follows we shall prove a theorem pertaining to the asymptotic development of the function F (z) defined by the series,

$$F(z)=\sum_{0}^{\infty}\frac{h(n)\cdot z^{3n}}{\Gamma(n+a_1)\cdot\Gamma(n+a_2)\cdot\Gamma(n+a_3)}.$$

The asymptotic expansion is based on the three Theorems given below§:

Theorem I. If the co-efficient $g(n)$ of the power series

$$f(z)=\sum_{0}^{\infty}g(n)\cdot z^{n};\ \text{radius of convergence}>0 \qquad (1)$$

may be considered as a function $g(w)$ of the complex variable $w=x+iy$ and as such satisfies the two following conditions when considered throughout any arbitrary right half plane $x>x_0$:

§ See *Asymptotic Developments*, Ford, pp. 4, 30.

(*a*) is single valued and analytic,

(*b*) is such that for all $|y|$ sufficiently large one may write

$$|g(x+iy)| < K \cdot e^{\epsilon|y|} \tag{2}$$

where ϵ is an arbitrarily small positive quantity given in advance and where K depends only upon x_0 and ϵ, then the function $f(z)$ defined by (1) is analytic throughout any sector S (vertex at the origin) of the z-plane which does not include the positive half of the real axis and $f(z)$ within S is developable asymptotically as follows:—

$$f(z) \sim -\sum_{1}^{\infty} g(-n)/z^n \tag{3}$$

Theorem II. If condition (*b*) stated in Theorem I is changed to (*c*) $g(w)$ is such that for all $|y|$ sufficiently large one may write

$$|g(x+iy)| < K \cdot e^{(\pi+\epsilon)|y|}$$

other condition remaining the same, then the function $f(z)$ defined in (1), when considered throughout any sector S (vertex at the origin) of the z-plane which does not contain the negative half of the real axis, may be expressed in the form

$$f(z) = \int_{-l-\frac{1}{2}}^{\infty} g(x) \cdot z^x \, dx - \sum_{1}^{l} \frac{g(-n)}{z^n} + \xi_l(z) \tag{5}$$

in which 1 is any positive integer ≥ 1 and

$$\lim z \to \infty \; z^1 \, \xi_l(z) = 0.$$

Theorem III. Let $P(w)$ be a function of the complex variable $w = x + iy$, which is single valued and analytic throughout the half plane $x > x_0$, where x_0 has some negative value previously assigned; also suppose that as $w \to \infty$ along any ray emanating from the origin upon which $-\pi/2 < \arg w < \pi/2$, the same function satisfies the following condition, in which c is a constant whose value is independent of the ray selected,

$$\lim w \to \infty \; P(w) = c$$

then, as $z \to \infty$ along any ray emanating from the origin in the z-plane upon which $-\pi/2 < \arg z < \pi/2$, we shall have,

$$\lim_{s \to \infty} e^{-s} \cdot \int_{0}^{\infty} \frac{P(x) \cdot z^{x-1}}{\Gamma(x)} \, dx = c \tag{6}$$

where the integration is understood to be along the real axis.

Using these three theorems we proceed to prove:—

Theorem IV. Let F (z) be a function of the complex variable z defined by the series

$$F(z)=\sum_{0}^{\infty}{}' \frac{h(n)}{\Gamma(n+a_1)\cdot\Gamma(n+a_2)\cdot\Gamma(n+a_3)}\cdot z^{3n} \tag{7}$$

in which a_1, a_2, a_3 are constants and in which $h(n)$ may be regarded as a function $h(w)$ of the complex variable $w=x+iy$ and as such satisfies the two following conditions:—

(a) $h(w)/\Gamma(w+a_1)\cdot\Gamma(w+a_2)\cdot\Gamma(w+a_3)$ is a single valued, analytic function of w throughout the finite w-plane,

(b). $h(w)$ is such that, when considered for values of w of large modulus lying in the right half plane $R(w)=x>x_0$ where x_0 is some assignable number, it may be expressed in the form

$$h(w)=\sum_{i=0}^{s+1}{}' \frac{b_i\cdot\Gamma(w+k)}{\Gamma(w+k+i)}+\frac{\delta(w,s)\cdot\Gamma(w+k)}{\Gamma(w+k+s+1)} \tag{8}$$

in which the b_i's are constants and $\lim_{w\to\infty}\delta(w,s)=0 \qquad (s=0,1)$.

Then F (z) has the following asymptotic development

$$F(z)\sim\frac{e^{3z}}{2\pi\cdot\sqrt{3}\cdot z^{p}}\left[\sum_{0}^{\infty}{}'\frac{C_m}{(3z)^m}\right],\quad -\pi/6<\arg z<\pi/6$$

where
$$p=a_1+a_2+a_3-2. \tag{9}$$

*Proof.** Let $f(z)$ be the function defined by the series

$$f(z)=\sum_{0}^{\infty}{}' g(n)\cdot z^n, \tag{10}$$

where
$$g(n)=\frac{h(n/3)}{\Gamma(n/3+a_1)\cdot\Gamma(n/3+a_2)\cdot\Gamma(n/3+a_3)}. \tag{11}$$

We have

$$F(z)=\tfrac{1}{2}\left[f(z)+f(z\cdot e^{2\pi i/3})+f(z\cdot e^{-2\pi i/3})\right. \tag{12}$$

so that our problem reduces to that of obtaining asymptotic expansions for the series occurring in (12).

$g(n)$ satisfies the conditions given in Theorem I and hence for all z of large modulus lying upon any ray for which $\pi/2<\arg z<3\pi/2$,

$$f(z)\sim-\sum_{-1}^{\infty}{}'\frac{h(-n/3)}{\Gamma(-n/3+a_1)\cdot\Gamma(-n/3+a_2)\cdot\Gamma(-n/3+a_3)\cdot z^n} \tag{13}$$

* The method of proof closely follows that adopted by Walter B. Ford in *Asymptotic Developments*, pp. 73.

$g(n)$ also satisfies the condition given in Theorem II and hence for all z of large modulus lying on any ray for which $-\pi < \arg z < \pi$,

$$f(z) = Q(z, l) - \sum_{1}^{l} \frac{h(-n/3)}{\Gamma(-n/3+a_1)\cdot\Gamma(-n/3+a_2)\cdot\Gamma(-n/3+a_3)\cdot z^n} + \epsilon_1(z) \quad (14)$$

where

$$Q(z, l) = \int_{-l-\frac{1}{2}}^{\infty} \frac{h(x/3)\cdot z^x}{\Gamma(x/3+a_1)\cdot\Gamma(x/3+a_2)\cdot\Gamma(x/3+a_3)}\, dx \quad (15)$$

and $\lim z \to \infty\ z^l \cdot \epsilon_1(z) = 0$

l being any arbitrarily large positive integer.

In order to study the behaviour of $Q(z, l)$ when z is large let us put

$$p = a_1 + a_2 + a_3 - 2 \quad (16)$$

and then write (15) in the form

$$Q(z, l) = \int_{-l-\frac{1}{2}}^{\infty} \frac{\Gamma(x+p+1)\cdot h(x/3)}{\Gamma(x/3+a_1)\cdot\Gamma(x/3+a_2)\cdot\Gamma(x/3+a_3)} \cdot \frac{z^x\, dx}{\Gamma(x+p+1)}. \quad (17)$$

Let us change the variable from x to t by the transformation $x+p=t$. Then we have

$$Q(z, l) = \int_{p-l-\frac{1}{2}}^{\infty} \frac{\Gamma(t+1)\cdot h\left(\frac{t-p}{3}\right)}{\Gamma\left(\frac{t-p}{3}+a_1\right)\cdot\Gamma\left(\frac{t-p}{3}+a_2\right)\cdot\Gamma\left(\frac{t-p}{3}+a_3\right)} \cdot \frac{z^{t-p}\, dt}{\Gamma(t+1)} \quad (18)$$

in which the path of integration extends from the point $t = p - l - 1/2$ to infinity in the direction of the positive real axis of t.

Now,

$$\Gamma(t+1) = \frac{3^{t+1/2}}{2\pi}\, \Gamma(t/3+1/3)\cdot\Gamma(t/3+2/3)\cdot\Gamma(t/3+1).$$

Using this result in (18)

$$Q(z, l) = \frac{\sqrt{3}}{2\pi z^p} \int_{p-l-\frac{1}{2}}^{\infty} \frac{H(t)\cdot h\left(\frac{t-p}{3}\right)}{1\ (t+1)}\, (3z)^t\, dt$$

in which

$$H(t) = \frac{\Gamma(t/3+1/3)\cdot\Gamma(t/3+2/3)\cdot\Gamma(t/3+1)}{\Gamma\left(\frac{t-p}{3}+a_1\right)\cdot\Gamma\left(\frac{t-p}{3}+a_2\right)\cdot\Gamma\left(\frac{t-p}{3}+a_3\right)}.$$

Using Stirling's formula and recalling (16), H (t) can be expanded as a factorial series. Hence, using (8) we write

$$\mathrm{H}(t)\cdot h\left(\frac{t-p}{3}\right) = \sum_{0}^{s} \mathrm{C}_n \frac{\Gamma(t+1)}{\Gamma(t+n+1)} + \frac{\sigma(t,s)\cdot\Gamma(t+1)}{\Gamma(t+s+1)}$$

where the C_n's are constants and $\sigma(t, s) = 0 \cdot (s = 0, 1, 2 \cdots)$ as $t \to \infty$ along any ray from $t = 0$ in the positive half of the t axis.

Hence we write

$$\mathrm{Q}(z, l) = \frac{\sqrt{3}}{2\pi z^p}\left[\sum_{0}^{s} \mathrm{C}_n \int_{p-l-\frac{1}{2}}^{\infty} \frac{(3z)^t}{\Gamma(t+n+1)}\, dt + \int_{p-l-\frac{1}{2}}^{\infty} \frac{\sigma(t, s)\,(3z)^t}{\Gamma(t+s+1)}\, dt\cdot\right. \tag{19}$$

Making use of Theorem II we get,

$$e^{3z} = \int_{p+n-l-\frac{1}{2}}^{\infty} \frac{(3z)^w}{\Gamma(w+1)}\, dw - \sum_{1}^{\mathrm{L}} \frac{1}{\Gamma(1-n)\, z^n} + \delta(z, \mathrm{L})$$

where

$$\lim z \to \infty\ z^{\mathrm{L}}\cdot\delta(z, \mathrm{L}) = 0 \text{ and } -\pi < \arg z < \pi,$$

and L denotes the greatest integer in $l - n - \mathrm{R}(p) - 1/2$, the path of integration extending to infinity in the direction of the positive real axis of w. By proper choice of l, L may be made independent of n and we then get

$$\lim z_s \to \infty\ z^n\cdot\delta(z, \mathrm{L}) = 0 \text{ for } n = 0, 1, 2, \cdots$$

Hence we get

$$\int_{p-l-\frac{1}{2}}^{\infty} \frac{(3z)^t}{\Gamma(t+n+1)}\, dt = \frac{e^{3z}}{(3z)^n}\,[1 + \theta(z, \mathrm{L})]$$

where $\lim_{z\to\infty} z^n\cdot\theta(z, \mathrm{L}) = 0$ for $n = 0, 1, 2, \cdots$

Thus the sum in (19) reduces to

$$e^{3z}\left[\sum_{1}^{s} \frac{\mathrm{C}_n}{(3z)^n} + \frac{\mathrm{C}_s\,\theta(z, \mathrm{L})}{(3z)^s}\right].$$

Also applying Theorem III the integral in (19) may be shown to be zero. Thus

$$\mathrm{Q}(z, \mathrm{L}) = \frac{\sqrt{3}\, e^{3z}}{2\pi z^p}\left[\sum_{0}^{s} \frac{\mathrm{C}_n}{(3z)^n} + \frac{\eta(z, s)}{(3z)^s}\right]$$

where $\lim_{z\to\infty} \eta(z, s) = 0$.

Using this result in (14) we finally get

$$f(z) \sim \frac{\sqrt{3}\, e^{3z}}{2\pi z^p} \Sigma \frac{\mathrm{C}_n}{(3z)^n} - \Sigma \frac{h(-n/3)\quad z^{-n}}{\Gamma(a_1 - n/3)\cdot\Gamma(a_2 - n/3)\cdot\Gamma(a_3 - n/3)}$$

when $-\pi/2 < \arg z < \pi/2$. Further we note that

$$z^{-p}\, e^{-3z} \cdot \Sigma \frac{h(-n/3)}{\Gamma(a_1 - n/3)\cdot \Gamma(a_2 - n/3)\cdot \Gamma(a_3 - n/3)\cdot z^n}$$

can be developed asymptotically in the form

$$0 + \frac{0}{z} + \frac{0}{z^2} + \cdots$$

when $-\pi/2 < \arg z < \pi/2$. Thus we finally get

$$f(z) \sim \frac{\sqrt{3}\, e^{3z}}{2\pi\, z^p} \sum_0^\infty \frac{C_n}{(3z)^n}.$$

Substituting $z \cdot e^{2\pi i/3}$ and $z \cdot e^{-2\pi i/3}$ for z in (20) and summing the three expressions so obtained we get

$$F(z) \sim \frac{e^{3z}}{\sqrt{3}\, 2\pi\, z^p} \sum_0^\infty \frac{C_n}{(3z)^n}.$$

It will be clear that the region for which the expansion remains valid is $-\pi/6 < \arg z < \pi/6$.

The series (20), (21) and (22) required in the body of the paper are only particular cases of $F(z)$. Thus to get the asymptotic expansion of series in (20), we have

$$F(z) = \sum_p^\infty \frac{z^{3n}}{n\cdot \Gamma(n+1)\cdot \Gamma(n+1)\, \Gamma(n+1)}$$

Here

$h(n) = 1/n$, $a_1 = a_2 = a_3 = 1$, and hence $p = 1$.

$$H(t) = \frac{\Gamma(t/3 + 1/3)\cdot \Gamma(t/3 + 2/3)\cdot \Gamma(t/3 + 1)}{\Gamma(t/3 + 2/3)\cdot \Gamma(t/3 + 2/3)\cdot \Gamma(t/3 + 2/3)}.$$

Now

$$\log \Gamma(x+h) = \tfrac{1}{2} \log 2\pi + x \log x - x + B_1(h)\cdot \log x - \Sigma (-1)^n \frac{B_{n+1}(h)}{n(n+1)\, x^n}$$

where B's are Bernoulli's polynomials of order n. Hence we get

$$\log H(t) = \frac{B_2(1/3) + B_2(1) - 2B_2(2/3)}{1\cdot 2\cdot t/3} + 0(1/t^2).$$

Using

$$B_2(h) = h^2 - h + 1/6$$

we get

$$\log H(t) = 1/3t + 0(1/t^2).$$

Hence

$$H(t) = e^{1/3t + 0(1/t^2)} = 1 + 1/3t + 0(1/t^2).$$

Therefore

$$H(t)\, h\left(\frac{t-1}{3}\right) = \frac{3}{(t-1)} + \frac{1}{t(t-1)} + 0\,(1/t^3).$$

Converting the above into factorial series we get

$$H(t)\, h\left(\frac{t-1}{3}\right) = \frac{3}{t+1} + \frac{7}{(t+1)(t+2)} + 0\,(1/t^3).$$

Thus

$$C_0 = 0,\ C_1 = 3,\ C_2 = 7, \text{ etc.}$$

Hence

$$F(z) \sim \frac{e^{3z}}{\sqrt{3}\, 2\pi z}\left[\frac{1}{z} + \frac{7}{9z^2} + 0\,(1/z^3)\right]$$

Similarly the asymptotic expansions for (21) and (22) are obtained.

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FLUCTUATIONS OF LIGHT INTENSITY IN CORONÆ FORMED BY DIFFRACTION

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(Communicated by Sir C. V. Raman, Kt., F.R.S., N.L.)

1. Introduction

The problem of determining the resultant of the secondary radiations emitted by a cloud of similar particles under the action of a primary wave is an important one in the field of optics. It might be supposed that when there are a large number, n, of such particles, distributed at random, the resultant intensity is just n times that due to a single particle. The late Lord Rayleigh (1871), however, showed that this is far from being the case, and that the resultant in any particular trial may be anywhere between o and n^2, and does not show any tendency to close upon the value n. He has also investigated (1880) the probability that the resultant intensity may lie between assigned limits of magnitude, and has derived an expression for the distribution of the resultant intensity.

In a general way, the existence of such fluctuations of intensity is illustrated by the well-known experiment of using a glass plate on which lycopodium powder is dusted as the diffraction screen, and viewing a source of light through it. The effects observed depend greatly on the size of the source and its spectral nature. Even as early as 1877, Exner observed the existence of radial streaks such as those shown in Fig. 3 (*a*), Plate I, when the corona is seen in white light. More recently, Laue (1916, 1917) and de Haas (1918) have observed the fluctuations in monochromatic light. In this case, the radial fibres reduce to a "mottled structure" of the type shown in Fig. 3 (*b*).* Laue 's studies led him to suppose that the phenomena could not be completely explained on the basis of classical wave-optics. This contention was disputed by de Haas, who performed further experiments supporting the view that the phenomena are explicable on wave-principles.

In this paper, some theoretical considerations are presented regarding the nature of the fluctuations, and experiments performed with a view to

* This will hereafter be referred to also as the "fine-structure" or "structure" of the corona.

substantiate the theory are also described. It is found, as is shown below, that de Haas's explanation of the phenomena is inadequate, and does not go to the root of the problem. In particular, the Rayleigh statistical law of distribution of intensity has been quantitatively verified for the first time, making use of the fluctuations of intensity in the corona as the basis for measurement.

2. *The Nature of the Fluctuations in Monochromatic Light*

de Haas's explanation of the mottled structure consists in considering every pair of particles to give rise to a set of interference fringes crossing the field. These are superposed, and produce fluctuations of intensity. This explanation, however, is wholly inadequate. In fact, when the number of particles is very large, one must take the aggregate effect of *all* the particles into account and not merely the effect due to pairs of particles. Each particle gives rise to a corona of its own, and the resultant intensity in any direction is due to the collective action of the wave-fronts diffracted by all the particles in that direction.

The failure of the de Haas point of view may be brought out strikingly by the following analogy. Suppose we are considering the opposite case of a regular square diffraction grating, formed for example by a square mesh of wires. In this case, one may take each pair of meshes in the grating, and imagine it as giving rise to a set of interference fringes of the type imagined by de Haas. But, such a method gives us no idea at all of what the nature of diffraction pattern due to the complete grating would be. The latter could be determined only by taking the waves diffracted by all the meshes simultaneously into account and finding their total effect. In those directions in which all these waves reinforce, we get bright spots, while elsewhere we have dark areas.

In the same way, with the lycopodium powder also, one has to consider the waves diffracted by all the particles together. Each particle becomes the centre of secondary radiation, and in those directions in which the phase relations between the waves diffracted by the particles happen to be such that there is a large co-operative effect, there will be bright spots. Since the positions of the particles on the screen are unknown, one cannot determine the positions of these maxima of intensity; but it is easily seen that there must be a large number of such spots irregularly arranged in the field of view.

We now proceed to show that these spots are in fact as sharply defined as the original image of the source formed by a lens whose aperture is the same as that of the diffracting screen. Consider a point P in

the focal plane of the camera where due to the co-operative action of the waves from the various particles, we have a large intensity, say I_P. The amplitude at this point will be $u_P = \sum_1^n e^{ip_m}$ where p_m is the phase of the mth particle on the screen, and the summation is done for all the n particles present, it being assumed that the amplitude due to each particle is unity. Therefore,

$$I_P = (\sum_1^n \cos p_m)^2 + (\sum_1^n \sin p_m)^2 = n + \sum_{r=1}^n \sum_{s=1}^n \cos (p_r - p_s). \quad (1)$$

Consider now a neighbouring point P′. At this point, the phases of the waves from the various particles would be altered. Let the alteration of phase for the mth particle be δ_m. Then, the amplitude at P′ is

$$u_{P'} = \sum_1^n e^{ip_m} e^{i\delta_m}, \text{ and the intensity is}$$

$$I_{P'} = \left[\sum_1^n \cos (p_m + \delta_m)\right]^2 + \left[\sum_1^n \sin (p_m + \delta_m)\right]^2. \quad (2)$$

This can be expressed in the form

$$I_{P'} = n + \sum_r \sum_s \cos (\delta_r - \delta_s) \cos (p_r - p_s) + \sum_r \sum_s \delta_s \sin (p_r - p_s).$$

If the δ's are small, this can be put in the form

$$I_{P'} = \left[n + \sum_r \sum_s \cos (p_r - p_s)\right] + \sum_r \sum_s \delta_r \delta_s \cos (p_r - p_s) + \sum_r \sum_s \delta_s \sin (p_r - p_s). \quad (3)$$

The second term is negligible, since it involves second order terms, so that

$$I_{P'} - I_P = \sum_{r=1}^n \sum_{s=1}^n \delta_s \sin (p_r - p_s). \quad (4)$$

Now, $\sin (p_r - p_s)$ is of the same order of magnitude as $\cos (p_r - p_s)$, so that $\Sigma\Sigma\, \delta_s \sin (p_r - p_s)$ is of a lower order compared with $\Sigma\Sigma \cos (p_r - p_s)$ which is the order of I_P. Thus, if δ is small, $(I_{P'} - I_P)$ is small compared with I_P.

Now, if we denote by $\delta\phi$ the angular separation of P and P′, then the largest value of δ_r or δ_s is given by $\pi b\, \delta\phi/\lambda$, where b is the width of the diffraction screen, and λ the wave-length of light. It is evident from (4) that $I_{P'}$ does not sensibly differ from I_P over a range of values of $\delta\phi$ within which the largest of the δ's does not exceed a fraction of 2π, and over this range of angles, the large intensity at P will persist. Putting the maximum value of the δ's for this to happen as, say, $\pi/4$ one gets the width of the bright spot as

$$\delta\phi = \frac{1}{4} \frac{\lambda}{b}. \quad (5)$$

Thus, the angular width of the bright spot is of the order of λ/b. Now, it is well known that the diffraction image due to a lens of aperture b also extends over an angle of the order of λ/b, so that the bright spot at P will be of the same sharpness as an image of the source formed by a lens of aperture b.

The argument used in the above discussion is, however, not restricted to the case when the intensity at P is a maximum. Actually, it is true whatever may be the intensity at P, so that one sees that the corona must actually consist of spots whose extension is of the same size as that of the image of the source. We have proved above that these images would not be spread out by an extent more than the aperture of the lens, so that we must expect the fine-structure to consist of sharp and well-defined images of the original source distributed at random. They are in fact the spectra formed by the irregular arrangement of the sources of secondary radiation produced by the particles.

Another interesting result, regarding the number of such spots in the field of view, comes out of the above discussion. As already remarked, the extent of the individual spots is given by the width of the aperture. Thus, we may divide the whole field of view into a number of areas, each of which is occupied by one spot. Now, on increasing the aperture, the area occupied by a spot decreases, so that more spots must appear in the field of view. To observe this phenomenon, the source of light must be small enough, so that the spots do not sensibly overlap. Otherwise, the fluctuations of intensity would all be wiped out by the overlapping, and the individual spots could not be discriminated. Under favourable conditions, however, the argument shows that the density or the number of spots per unit area must depend on the aperture employed, increasing with increase of its dimensions.

3. *Experimental Confirmation*

The verification of the above two deductions from the theory was done by the following experimental arrangement. The source of light, S (Fig. 1) was a pinhole, or an aperture of any size and shape as desired, which was illuminated by the filtered mercury radiation of 5461 A. U. At a certain distance from the source was placed a glass plate G, on which lycopodium powder was dusted. The resulting diffraction pattern could be brought to a focus on a plate C by a lens L. An aperture AB was placed in front of the lens, and its size and position could be adjusted. When necessary, the camera could be removed and the pattern directiy viewed with the eye.

Initial visual observations using a small circular hole as the source showed that the spots in the corona are also all circular in shape and of the

same size as the original source. On increasing or decreasing the size of the pinhole, the spots in the field of view also correspondingly became larger or smaller showing that they are images of the source. But the most convincing proof of this fact was obtained by using a triangular slit as the source, when every one of the spots assumed a triangular shape, and besides had the same size and orientation as the source. Photographs were then taken to illustrate this fact. They are reproduced in Fig. 4 (*a*) and (*b*), Plate I. Fig. 4 (*a*) was taken with a circular pinhole as the source, and Fig. 4 (*b*) with a triangular slit. They show in an unmistakeable manner that the spots are in reality images of the source.

The second conclusion from the theory was also verified by observation. On increasing the area of the aperture, it was found that the number of spots in the field of view increased, and *vice versa* as demanded by the theory.

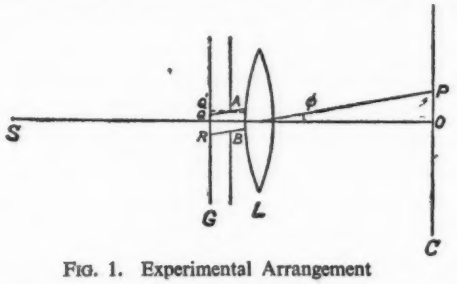

FIG. 1. Experimental Arrangement

During these experiments, a few other interesting phenomena were observed which also find a ready explanation on the idea that the spots are the spectra produced by the collective diffraction by all the particles.

(*a*) If one moves the screen containing the lycopodium powder keeping the eye fixed on the source, then the ring system is not found to undergo any change, but the fine-structure appears to move relative to the pattern of rings in the same direction as the motion of the screen. *Vice versa*, keeping the screen fixed, if one moves the eye, all the while looking at the source, then the fine-structure appears to move in a direction opposite to the motion of the eye. This effect is only the converse of the previous one. Also, if the screen is rotated, the fine-structure appears to rotate in the same direction.

These effects can be explained as being purely due to the geometry of the arrangement. Since the pupil of the eye is small, the whole of the screen does not contribute to the diffraction pattern nor is every portion of the pattern produced by the same portion of the screen. If we consider the corona in a direction ϕ (Fig.1), the intensity in this direction is due only to the waves diffracted by the region QR of the screen. Hence, if the screen is moved up, the

same portion QR is now responsible for the corona in a direction ϕ' parallel to Q'A. This happens for every portion of the screen, so that the fine-structure as a whole appears to move in the same direction as the screen relative to the rings. The effect of rotation is too obvious to need an explanation.

(*b*) During these experiments, it was found that the fine-structure not only moved bodily in the same direction as the motion of the screen, but that there were some internal changes as well. To follow these, the bodily motion was avoided in the following way. The aperture AB was placed in contact with the screen, and the lens L of the camera was large enough so that the whole diffraction pattern was caught by it. In this way, the difficulty of different portions of the screen giving rise to different portions of the corona was overcome. The whole of the screen was always operative. Now, on moving the screen in its own plane, the fine-structure pattern at the focal plane of the lens did not show any bodily movement; but there was an internal rearrangement of the spots.

This phenomenon could be explained as due to the fact that spherical waves are incident on the screen so that, on moving the screen in its own plane, the phase of the wave falling on each particle is altered. Thus, the phase relations between the waves emitted by different particles are changed, which leads to a change in the pattern of the spectra given by the particles and hence to a random rearrangement of the spots.

It is obvious that these internal changes must vanish if the incident wave is plane, for then a movement of the plate in its own plane should produce no change in the phase relationship between the different particles. This was tested out experimentally and it was found that with parallel light incident on the screen, the fine-structure pattern at the focal plane of the lens was entirely unaffected by moving the screen in its own plane. This shows that the explanation given above for the internal motion of the spots is correct.

(*c*) The phenomenon of the internal rearrangement of the spots can be observed with the eye also, if the aperture AB is made sufficiently small. In this case, on moving the aperture over the screen, different portions of the screen are operative, and since the arrangement of the particles in these are different, the spots in the field of view exhibit a beautiful "dance". The appearance is somewhat similar to that of the field of a spinthariscope, with bright spots appearing and disappearing at random positions.

4. *Fluctuations in White Light*

We have already shown how, in monochromatic light, the fine-structure consists of a series of images of the source. We now proceed to investigate

what happens if white light is used. Suppose that, with monochromatic light of wavelength λ, there is a bright spot at P, in the direction ϕ (Fig. 1). In the diffracting screen, draw the X-axis parallel to OP, and the Y-axis perpendicular to it, and let (x_m, y_m) be the co-ordinates of the mth particle on the screen with respect to these axes of co-ordinates. Then, (supposing the incident wave to be plane) the intensity in the direction ϕ is equal to

$$\left| \sum_{m=1}^{n} e^{ikx_m \sin \phi} \right|^2, \tag{6}$$

where k stands for $2\pi/\lambda$. Now, supposing the wavelength of the light to be changed to λ', and that $k' = 2\pi/\lambda'$, then it is readily seen from the above expression that the value of the intensity is unaltered provided we change ϕ to ϕ', such that

$$k \sin \phi = k' \sin \phi', \text{ or } \sin \phi/\lambda = \sin \phi'/\lambda'. \tag{7}$$

Hence, the bright spot, which was formerly at P, would now appear at another point P′ in the direction ϕ' given by expression (7) such that P′ lies along OP. This argument also is not restricted to the case when P is a bright spot, but is quite general. Hence, with an increase in the wavelength of the light, the whole pattern expands radially so that to every point P in the first case there is one, P′, in the second, such that

$$\frac{OP}{\lambda} = \frac{OP'}{\lambda'}. \tag{8}$$

If white light is used, there will be a range of wavelengths, so that there will be no discrete spots, but each spot will be spread out into a radial streak, whose colour is violet at the inner end and red at the outer end. The length of every such streak will be directly proportional to its distance from the centre, the streaks further away from the centre being longer.

As already remarked, such radial fibres were actually observed in white light by Exner. The author has observed these carefully and has found that one can actually follow the spectral colours in the order violet to red in any single fibre, as one goes away from the centre. Very close to the centre of the field, however, one can observe a few white spots, since the dispersion in them is very small on account of their proximity to the centre. The length of the fibres (the distance from the violet to the red end) is found to be proportional to their distance from the centre, and the length of any fibre at a certain distance is found to be a constant, within the limits of the experimental error.

In order to further demonstrate the correlation between the spots in monochromatic light, and the fibres in white light, the following experiment

was performed. A pointolite lamp was used to illuminate a fine pinhole, and a mercury green filter supplied by Adam Hilger & Co., was interposed. This transmitted only two bands, one in the green and the other in the red. The fine-structure now presented a beautiful appearance. The pattern was filled with red and green spots; but every green spot was accompanied by a red one, the two being along a radius, and the red spot being the outer one. It was also verified that the ratio of the distances of the red and the green spots from the centre was a constant, and was equal to the ratio of the mean wavelengths of the red and green transmission bands.

5. *Verification of the Rayleigh Law of Fluctuations*

Lord Rayleigh (1880) derived an expression for the distribution of intensity in the resultant due to n vibrations whose phases are at random. If the amplitude of a vibration is taken as unity, and as a special case, if the phases are assumed to have only either of values 0 or π, then it follows directly from Bernoulli's theorem that the chance of a positive amplitude between x and $x+\delta x$ is $\frac{1}{\sqrt{2\pi n}}\, e^{-x^2/2n}\, \delta x$. In the more general case of arbitrary phase, the probability that the resultant amplitude may lie between r and $r+\delta r$ comes out as

$$\frac{2}{n}\, e^{-r^2/n}\, r\, \delta r. \tag{9}$$

From this, it follows that the probability that the intensity may be between I and $\mathrm{I}+\delta \mathrm{I}$ is

$$p\,(\mathrm{I})\,\delta \mathrm{I} = \frac{1}{n}\, e^{-\mathrm{I}/n}\, \delta \mathrm{I}. \tag{10}$$

We can put this in a more convenient form. From (10), it can be shown that the average value of the intensity is n. Expressing I as a fraction f of the average value, *i.e.*, putting $\mathrm{I}/n = f$, (10) reduces to

$$p\,(f)\,\delta f = e^{-f}\, ef. \tag{11}$$

This gives the probability that the resultant intensity may lie between the fractions f and $f+\delta f$ of the average, and is the same function for all values of n. It is a maximum for $f=0$, and decreases as f increases.

This law can be verified by making use of the fine-structure in the corona. As already said, the fine-structure is produced as a result of the interference of the light diffracted by all the powder grains. The relative phases of these diffracted waves are not known but are distributed at random. Also, for different angles, ϕ, the phase relations are different, so that the whole pattern actually presents the effect of different combinations of these phase relations.

Hence, the resultant intensity in the pattern must show the same statistical law of fluctuations as demanded by Rayleigh's theory.

This was verified in the following way. A very fine pinhole illuminated by the mercury green radiation of 5461 Å.U. was used as a source of light, and a picture of the central portion of the corona was taken with an exposure of two hours on a Selochrome plate. To obtain a standard of intensities, a square grating was used instead of the lycopodium screen, and the same exposure was given for the grating also. The grating gave a spectrum, the intensities of whose spots are calculable. The plates for the two photographs were obtained by cutting a single quarter plate into two. Both were developed simultaneously in the same developing bath for the same time. Thus, a strictly comparable scale of intensities was obtained for determining the intensity of the spots in the corona. The photographs of the corona and the standard grating spectra are reproduced in Fig. 5 (*a*) and 5 (*b*), Plate I, respectively.

Now, in the corona, the average intensity falls away from the centre in the proportion $J_1^2(x)/x^2$ where $x = 2\pi a \sin(\phi)/\lambda$, a being the radius of the particle, ϕ the angle of diffraction, and λ the wavelength of the light. The corona taken in the present case extended upto a value of $x = 1{\cdot}5$, for which the average intensity was about half that at the entre. It was therefore divided into annular rings, the widths of which were all equal to $x = 0{\cdot}25$, by drawing circles of radii corresponding to $x = 0{\cdot}25$, $0{\cdot}5$ etc. Within each ring, the value of $J_1^2(x)/x^2$ did not vary much, and the average value for a ring was taken to be correct for that ring.

Using the grating spectrum, the intensity of whose spots could be calculated, an arbitrary scale of intensities was obtained. Then, the spots in each ring were classified into this scale, comparing them visually with the standard. The spots having an intensity less than 1 in the arbitrary scale, *i.e.*, those that could not be differentiated with the eye were found by calculating the total number of spots (dividing the area of the ring by the area of a spot) and subtracting from this the number of counted spots. Using this tabulated list, the average intensity (on the arbitrary scale) for that ring could be determined. Thus, the fraction of the total number of spots having an intensity between the fraction f_1 and f_2 of the average could be found. Dividing this by $(f_1 - f_2)$ we get the value of $p(f)$ at $(f_1 + f_2)/2$. Thus, $p(f)$ was evaluated for certain values of f. This was done for four rings, *viz.*, $x = 0{\cdot}25$ to $0{\cdot}50$, $0{\cdot}50$ to $0{\cdot}75$, $0{\cdot}75$ to $1{\cdot}00$, and $1{\cdot}00$ to $1{\cdot}25$. These were plotted in a graph and are reproduced in Fig. 2. The continuous curve represents the theoretical curve calculated from theory, and the experimental values are plotted by points.

A final average of all these four rings was obtained by dividing the intensities of the spots in each by the value of $J_1^2(x)/x^2$ for it, and then determining $p(f)$ and f taking them all into account. These are tabulated in the table below, and are represented by black dots in Fig. 2. It is clearly seen that the experimental points fit the theoretical curve very well, considering

f	$p(f)$	Theoretical	Difference
0·44	0·60	0·62	−0·02
1·29	0·33	0·28	+0·05
2·13	0·14	0·12	+0·02
3·45	0·02	0·03	−0·01
6·25	0·002	0·002	..

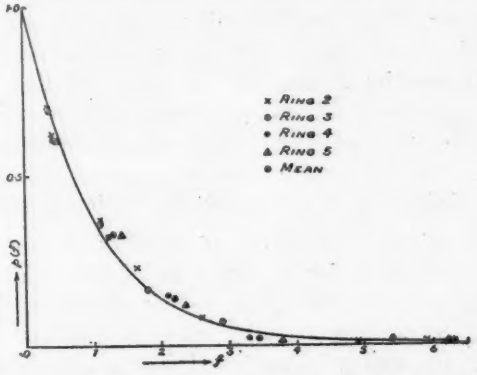

Fig. 2. Verification of Rayleigh's Statistical Law

that the comparison of intensities was done visually. Rayleigh's formula is therefore verified to be true for the distribution of intensity in the corona.

6. *Conclusion*

The above considerations regarding the fluctuations of intensity are true only for a static diffraction screen, *i.e.*, one in which the phase relations between the various particles is fixed in time. If the particles themselves are in motion, as in a gas or a cloud, then a continuous redistribution of phase takes place, and what one sees is the integrated effect of all these over a definite period. As Lord Rayleigh (1918) has shown, such a redistribution tends to make the resultant intensity (perceived as an average over a certain period) approach the average value n, this tendency being greater the larger the number of redistributions. Hence, for the light scattered by a gas, or a

cloud of particles, one is quite justified in regarding the scattered intensity in inclined directions as equal to n times that due to a single particle.

If the motion of the particles is slow, then the alteration in the position of the spots in the fine-structure will also be slow, and can be observed. Such a slow random motion takes place in Brownian movement, so that it must be capable of detection by this technique. This possibility, which was suggested by Prof. Sir C. V. Raman, is now under investigation.

I wish to express my sincere thanks to Prof. Sir C. V. Raman for suggesting the problem, and for the many helpful hints he gave during the investigation.

7. *Summary*

The fluctuations of light intensity in the diffraction corona produced by a large number of randomly distributed particles are investigated both theoretically and experimentally. The theoretical considerations show that the view put forward by de Haas is inadequate and that really the fluctuations arise owing to the interference of the waves diffracted by *all* the particles, which gives rise to a large number of sharp images of the source in monochromatic light. In white light, these spots must spread out, and produce radial streaks. These deductions from theory are all borne out by experiment.

Making use of the fluctuations of intensity in monochromatic light, verification has been made for the first time of Rayleigh's statistical law of distribution of intensity in the resultant of n vibrations of arbitrary phase. It is shown that the resultant intensity shows no tendency to close upon the average value, n, but that it is distributed over a wide range of values.

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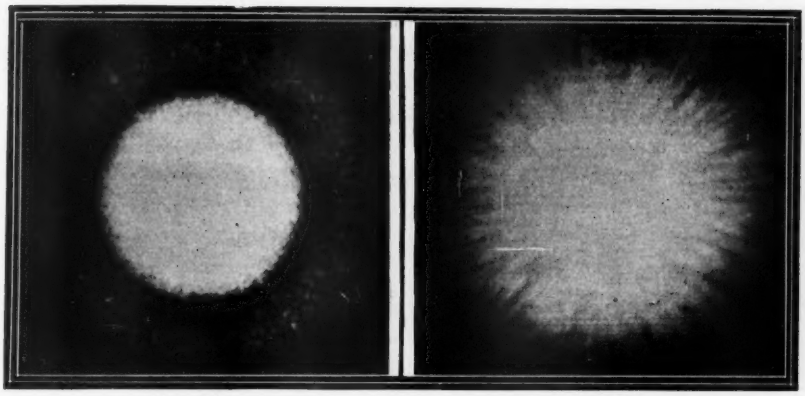

FIG. 3(*a*) FIG. 3(*b*)

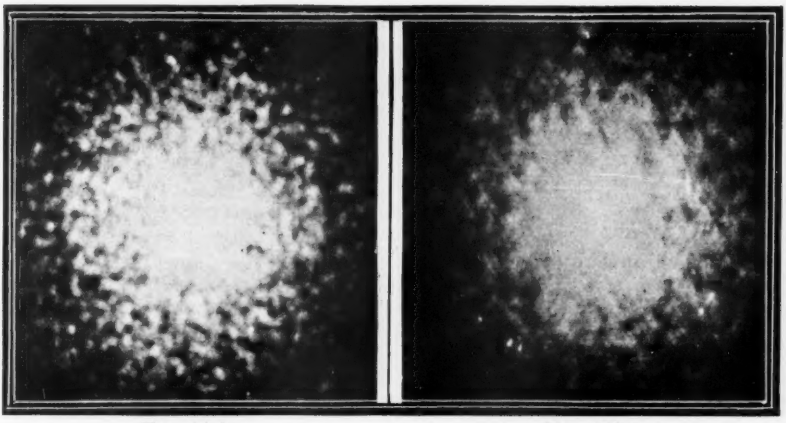

FIG. 4(*a*) FIG. 4(*b*)

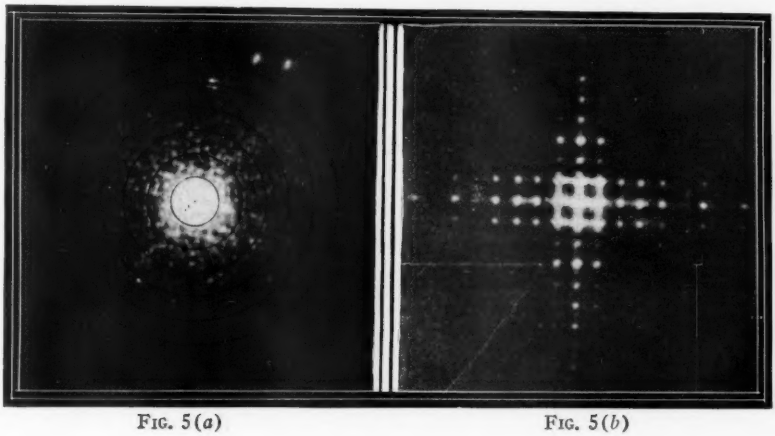

FIG. 5(*a*) FIG. 5(*b*)

CHEMICAL INVESTIGATION OF INDIAN FRUITS

Part IV. A Note on the Bitter Principle of a Variety of *Citrus limetta*

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A VARIETY of *Citrus limetta* available in the northern and north-western parts of India during the hot weather has certain peculiar characteristics. It is quite juicy and the juice when carefully sucked out is almost insipid having only a faint sweet taste. But if the fruit should be crushed leading to pressure on the rags, the juice becomes exceedingly bitter. The bitter principle is present mainly in the rags. The thin peel which is rather difficult to remove is not so bitter. The seeds are very small. The external appearance of the fruits is quite similar to that of the sweet variety of *Cirtus limetta* (mussambi) common in Central and Western India and both go by the same common name, Mitta. The rind is yellow to orange in colour and smooth.

A fruit weighs on an average 200 grams and the juice yield is 100 to 120 grams (50 to 60%). The peels constitute 12–15%, the fresh rags 25–30% and the seeds about 0·7% of the fruit. The results of analysis of juice (average of several experiments) is as below: Brix 8·3°, reducing sugars 6·3%, total sugars 6·3%, sucrose nil, acidity 0·01% as citric acid. Thus there is very little acid and though the sugar content is not very low, sucrose is absent and consequently sweetness is poor.

The bitter principle was extracted from the rags by repeated treatment with cold alcohol and the extract concentrated to small bulk. With a view to detect the existence of more than one crystalline entity, the solid matter was isolated in fractions and then purified. By the addition of an equal volume of water to the alcoholic concentrate fraction (I) was obtained. Concentration of the mother-liquor during which most of the residual alcohol was evaporated, yielded fraction (II). The final mother-liquor (M) was separately examined. When fraction (I) was extracted with excess of boiling acetone, most of it dissolved leaving behind a very small quantity of resinous matter. On allowing the solution to concentrate a crystalline solid was obtained (fraction I A). Addition of water to the mother-liquor yielded an amorphous solid (fraction I B). Fraction (II) was also soluble in acetone and from it fractions (II A) and (II B) were obtained.

Fraction (I A) was insoluble in water, sparingly soluble in alcohol and readily soluble in acetone. It was best crystallised from acetic acid from which it came out as colourless rectangular prisms and tablets. The substance charred and burnt when introduced into a flame and left no residue. It melted at 292–94° with decomposition. When an alcoholic solution of it was treated with magnesium and hydrochloric acid no red or orange colour was developed. A dilute aqueous alcoholic solution was quite bitter; $[\alpha]_D^{30°}$, $-115{\cdot}1°$ in acetone solution. From these properties it seemed to be limonin and this surmise was confirmed by careful comparison with an authentic sample of limonin obtained from the seeds of Indian shaddock[1] and determination of the mixed melting point. Fractions (I B), (II A) and (II B), when subjected to crystallisation, yielded the same product thereby showing that there was only one bitter principle in all of them.

The aqueous mother-liquor (M) was made 7% acid with sulphuric acid and boiled for 2 hours. To start with there was a clear solution and at the end a dark brown resinous solid was found to have separated out. It was almost insoluble in acetone and an alcoholic extract (sparingly soluble in alcohol) gave no colour with magnesium and hydrochloric acid. No crystalline substance could be isolated from it. Thus the absence of naringenin in the resinous matter and of naringin in the aqueous mother-liquor was estabilished.

Consequently the only crystalline bitter principle of the above variety of *Citrus limetta* is limonin. This bitter substance has been formerly found to occur mainly in the seeds of the lemon, oranges and shaddock and it is invariably accompanied by more or less quantities of the closely related compound, isolimonin.[2,1]

It seems to be now clear that these complex bitter compounds of unknown constitution contribute also to the bitterness of the rags and peels of certain types of oranges. Isolimonin has been found by Higby[3] to be the main bitter component of the peels and rags of American Navel oranges and limonin to be present in the pulp of Valencia oranges. The present note records a further case of this kind. The constitution of limonin is quite indefinite. But its formula seems to be definite as $C_{26}H_{30}O_8$. Since the number of carbon atoms is near those of sterols and resinols, its reactions with the special reagents of these groups of compounds has now been studied. With the Liebermann-Burchard reagent it yields a very pale yellow solution. On the other hand, with the Salkowski reagent, the chloroform layer is colourless with a weak bluish violet fluorescence and the sulphuric acid layer first turns

yellowish brown and in the course of a few minutes becomes deep brown red. These characteristics are different from those of sterols and of resinols.

Summary

A variety of *Citrus limetta* has been found to contain limonin as the only bitter principle of the rags. The characteristics of the fruit and its juice are described.

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PIGMENTS OF COTTON FLOWERS

Part IX. A Note on the Occurrence of Populnetin in Indian Cotton Flowers

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In the course of their study of the colouring matter of the flowers of *Gossypium indicum*, Neelakantam and Seshadri reported the isolation of a new non-glycosidic compound.[1] The total pigment of the flowers was separated into five fractions according to the general scheme and studied. The first alcohol fraction contained mainly gossypin, a complex glycoside of gossypetin and the second (aqueous) fraction gave a small amount of the new substance which was not given any name at that time since it could not be studied in detail and its individuality was not quite definite. In the course of our examination of various samples of *G. herbaceum* flowers this substance has been again met with in small quantities and hence it occurs in this source also. Its main characteristics are (1) stability to aerial oxidation in alkaline solution thus indicating that it is a flavone, (2) lack of any colour changes in buffer solutions of pH on the alkaline side, (3) dissolution in concentrated sulphuric acid forming a yellow solution with a bright green fluorescence, (4) absence of any precipitate when an alcoholic solution is treated with neutral lead acetate, (5) formation of a yellow precipitate with basic lead acetate and (6) absence of methoxyl in its composition. These seemed to indicate that it was probably populnetin[2] and the idea was supported by the fact that populnetin occurs along with herbacetin in the flowers of *Thespasia populnea*[3] and hence could be expected to accompany it in the Indian cotton flowers also. But the melting point of the substance was rather low and the results of analysis were not definite due probably to the presence of inseparable impurities and the existence of hydration which seemed to vary with different conditions. The preparation and study of the acetyl derivative were not more helpful. The quantity available after these experiments was too small for further repeated purification and a detailed study independently. Consequently it was subjected to complete methylation using an acetone solution and excess of dimethyl sulphate and dilute sodium hydroxide. The product could be crystallised from alcohol from which it came out in the form of rectangular plates which were almost colourless. In its melting point, reactions and analysis, it was found to be identical with a sample of tetramethyl populnetin (described below) obtained by methylating an

authentic sample of populnetin isolated from *T. populnea*. It appears that definite purification is effected during methylation leading to the isolation of a pure methyl ether. The non-glycosidic substance should therefore consist mostly of populnetin.

Methylation of Populnetin

The flavone (1 g.) was dissolved in acetone (40 c.c.) and treated alternately in small quantities with 10% aqueous sodium hydroxide (50 c.c.) and dimethyl sulphate (10 c.c.). Finally the medium was made alkaline by the further addition of the alkali (50 c.c.) with vigorous shaking. In an hour an almost colourless crystalline compound separated out. It was filtered and recrystallised from alcohol using a little animal charcoal. Under the microscope it appeared as rectangular plates which were almost colourless. On heating it shrank at 95–100° (dehydration) and melted at 164–66°. It was insoluble in dilute alkali and did not yield any colour with ferric chloride. Its solution in concentrated sulphuric acid had a weak green fluorescence. [Found in the air-dried sample: C, 60·6; H, 6·2, and loss (H_2O) on heating at 110° for 2 hours *in vacuo*, 9·2%. $C_{15}H_6O_2$ $(OCH_3)_4$, $2H_2O$ requires : C, 60·3; H, 5·8 and H_2O loss, 9·5%. Found in the dehydrated sample: C, 66·2; H, 4·9%; $C_{15}H_6O_2$ $(OCH_3)_4$ requires C, 66·6; H, 5·2%.] The mixed melting point with the methyl ether obtained from the sample derived from the cotton flowers was undepressed.

From the experiments described above it is clear that populnetin occurs free in the Indian cotton flowers along with gossypetin, herbacetin and quercetin which are present mostly as glycosides. Thus there is a further case of the association which was first found in the flowers of the *Thespasia populnea*. It has already been shown that the substance is a tetrahydroxy flavone having a hydroxyl in the 4′-position. The disposition of the other groups in the benzopyrone part is still a matter of investigation. Its occurrence along with compounds having the 5 : 7 : 8-orientation of hydroxyls seems to suggest a similar disposition of the hydroxyl groups in it also.

Summary

The new non-glycosidic substance obtained from the Indian cotton flowers has been shown to consist mostly of populnetin from a comparison of the methyl ethers. The characteristics of the methyl ether of populnetin are described.

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CHEMICAL EXAMINATION OF INDIAN ERGOT OF THE NILGIRIS

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Some time back Thomas and Ramakrishnan[1] described their very successful experiments on the production of ergot of rye in the Nilgiris. Mr. Thomas sent us a sample as early as October 1942. We intended to make a detailed study of it as the first sample grown under Indian conditions and obtain information regarding the influence, if any, of habitat on the chemical composition. Owing to serious difficulties which we then had of laboratory and library we could not undertake the complete examination immediately; but after a proximate analysis the material was defatted and preserved. Further examination was carried out in March 1943 and the results were not without interest. Meanwhile Mukherji and Dey[2] published their note on the assay of Indian ergot and expressed their opinion that the Nilgiris ergot was at least of the British Pharmacopœial quality, if not better. Our results indicate that our sample is really of very high quality comparing favourably with the richest ever produced in different parts of the world and hence they are presented here.

Proximate analysis of the entire drug by standard methods gave the following values; the values reported by previous workers and taken from Barger's book on 'Ergot and Ergotism'[3a] are given within brackets for comparison.

Moisture	7·9% (4·4 – 10·0)
Ash	3·0% (2·2 – 7·0 ; average 4·0)
Total Nitrogen	3·57%
Fat (Petroleum ether extract)	27·3% (21·0%)

The fat which was a liquid had the following characteristics ; the figures within brackets have the same significance as above.

Refractive index at 32° C. ..	–1·466 (1·4685 – 1·4739 at 20° C.)
Saponification value ..	196·2 (178·4 – 196·9)
Iodine value (1 – hour Wij's)	–72·9 (69·55–73·8)
Unsaponifiable matter ..	1·7% (0·35–1·04; average 1·0)
Hehner number	96·5 (96·0–96·3)

The rest of the sample of ergot was powdered, defatted thoroughly with petroleum ether (boiling range 40–50°) by percolation, dried in the air and preserved in an air-tight container. The alkaloidal assay was carried out by the method of Hampshire and Page[4] as described in Garratt's book *Drugs and Galenicals*.[5] 7·0 gm. of the defatted sample were taken for the assay. The rest of the procedure was in general according to the description given in the above-mentioned reference except that a slightly larger volume of 1% tartaric acid was used for extracting the alkaloids from ether solution and the final tartaric acid solution had to be rediluted to give an easily measurable intensity of colour with the special alkaloidal reagent prepared according to the 1936 Addendum to the 1932 B.P. The concentration of alkaloids was obtained by using the Lovibond Tintometer of the B.D.H. pattern for measuring the intensity of colour. When the colour was developed using 1 ml. of the above solution and 2 ml. of the reagent, 8 blue units on the Lovibond Tintometer were taken to represent a concentration of 0·0001 gm. of anhydrous ergotoxine per ml. (*vide* Garratt, p. 112). The following values were obtained: Total alkaloids as anhydrous ergotoxine 0·585%; water-insoluble alkaloids as anhydrous ergotoxine 0·417%; water-soluble alkaloids as ergometrine 0·090%. The last figure was calculated by using the relation obtained by Hampshire and Page (*loc. cit.*) between ergotoxine and ergometrine, *viz.*, that the colour equivalent of ergometrine is 1·86 times that of ergotoxine. All the above figures are with reference to the defatted ergot sample. The corresponding values calculated on the original ergot sample will be 0·425, 0·303 and 0·0654% respectively.

In the meanwhile the note of Mukherji and Dey (*loc. cit.*) appeared in *Current Science* reporting values as low as 0·1213% for total alkaloids and 0·0237% for water-soluble alkaloids, based on the results of analysis by the same chemical method of Hampshire and Page and confirmed by pharmacological assays. With a view to see if our high value was due to any errors in the technique or in the assumed relation between ergotoxine content and blue value the sample was reassayed according to the standard method of 1932 B.P. modified by the 1936 Addendum. However, in view of our previous high results only 2·0 gm. of defatted ergot powder was taken. The rest of the procedure was according to the B.P. The total volume of the tartaric acid solution used in the extractions of the alkaloid from ether solution finally amounted to 52 ml. and this solution was further diluted with an equal volume of 1% tartaric acid for convenient colour matching. The comparison was done against a freshly prepared solution of ergotoxine ethanesulphonate taken from a freshly opened sealed tube of the compound procured from Messrs. B.D.H. and a Duboscq type of colorimeter was employed. The

value for total alkaloids obtained by this method was 0·465% as anhydrous ergotoxine calculated with reference to the defatted ergot and 0·338% on the entire ergot. It is well known that the B.P. method of assay includes only a portion of the water-soluble alkaloids.

The standard solution of ergotoxine ethanesulphonate was also utilised for checking the accuracy of the tintometric colour relation with the alkaloid content. Careful readings showed that the tintometer used in the previous assay entirely satisfied the colorimetric relation. 0·0001 gm. of anhydrous ergotoxine contained in 1 ml. of solution gave with the standard reagent under standard conditions a colour whose blue component was 8 units; so that the previous assay using only the tintometer for the evaluation of the total and water-soluble alkaloids was entirely correct.

Thus it is obvious that the sample of ergot examined by us is very rich in alkaloid content. There is nothing improbable in this high value. In his well-known book Barger[3b] gives numerous data for the alkaloidal content of various samples as obtained by different workers. Therein values as high as 0·38 and 0·414% are found for certain samples. More recently Bekes[6] has reported a value as high as 0·74% obtained in the course of large-scale experiments on the intensive production of rye-ergot. The same author has also studied the alkaloidal content of individual sclerotia[7] and reported values as high as over 1% of total alkaloids with several samples. It should however be noted that variations are possible in the qualities of the samples depending on differences in the conditions of collection and preservation and this may account for the differences between our results and those of others.

In any project for the production of ergot adequate precaution has to be taken to prevent infected rye grains finding their way into those collected for food, since ergot is a poison. A reliable chemical test for the presence of ergot makes use of the colour reaction for sclererythrin which is a crimson violet colouring matter present in the walls of the cortical hyphæ, probably as the calcium salt. The test was carried out as below with the present sample of ergot: About 0·1 gm. of the powdered ergot was skaken with 5 ml. of ether and a few drops of dilute sulphuric acid for 5 minutes. The ether solution was then decanted, diluted to 10 ml. and half the volume treated with 2 ml. of a saturated solution of sodium bicarbonate. A deep violet colour was formed in the aqueous layer. This colour test shows that the Nilgiris ergot easily conforms to the ordinary requirements of ergot for purposes of testing food grains.

Summary

A sample of Indian ergot of the Nilgiris has been studied in detail. It contains remarkably high percentage of total and water-soluble alkaloids. With respect to other components it is normal.

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INTERFEROMETRIC STUDIES OF LIGHT SCATTERING IN BINARY LIQUID MIXTURES: PART I

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1. *Introduction*

The blue opalescence of binary liquid mixtures in the vicinity of their critical solution temperature has been the subject of numerous investigations in the past. Smoluchowski (1908) was the first to put forward a thermodynamic explanation of this phenomenon as due to optical inhomogeneity caused by spontaneous local fluctuations in concentration in the mixture. Einstein (1910) extended this theory with certain simplifying assumptions, namely (1) that the liquids are incompressible; (2) that their specific volumes are negligibly small in comparison with those of the saturated vapours emitted by them; and (3) that the latter can be treated as ideal gases and on this basis derived an expression for the intensity of the opalescent light. This theory predicts an infinite intensity of scattering and also complete polarisation of the scattered light at the critical solution temperature. Experimental investigations of the opalescent light by several authors however showed that as the critical solution temperature is approached neither does the intensity of scattering tend to infinity nor is the polarisation of the scattered light perfect. In order to explain these difficulties Ornstein and Zernike (1914, 16, 18, 26) put forward a modified theory which considered the mutual influence of fluctuations in composition in neighbouring volume elements and according to which the intensity assumes a finite value at the critical temperature and is proportional to λ^{-2} and not to λ^{-4} as given by the formula of Smoluchowski-Einstein.

In order to explain the persistence of the opalescent phenomenon over a wide range of temperatures accompanied by remarkable changes in intensity and state of polarisation Raman and Ramanathan (1923) revised Einstein's theory taking into account the compressibility of the mixture and the varying orientations of the molecules. According to them the total intensity of light scattered by liquid mixtures is the aggregate of the separate effects of (1) fluctuations in concentration of the mixture, (2) fluctuations of its density and (3) the varying orientations of the anisotropic molecules.

Einstein's theory ignores the latter two effects which become of increasingly greater importance relative to the first at temperatures above or below the critical solution temperature.

The earlier investigators in this field suggested as an explanation of the opalescence before the thermodynamic explanation was put forward by Smoluchowski that the liquid mixture in the vicinity of the critical solution temperature behaves as an emulsoid. That this suggestion is not altogether baseless has been shown by Krishnamurti (1929) who found in X-ray diffraction studies with liquid mixtures that there are in general two types of liquid mixtures, one giving two separate rings for the two components and the other giving mainly one ring. The partially miscible liquids below their critical temperatures are found to belong to the first type and completely miscible liquids to the second type. The tendency for the formation of two distinct rings is explained by him by postulating the formation of large molecular clusters of the individual components. Recently Krishnan (1935) by means of a delicate optical method has shown that the molecules of critical composition mixtures in the neighbourhood of their critical solution temperatures have got a tendency to group themselves into clusters and that the finite value of the depolarisation of the opalescent light arises from the fact that the size of the clusters become comparable with the wavelength of light. Other experimental evidence such as the abnormal increase in the coefficient of viscosity and the thermal coefficient of magnetic and electric birefringence at the critical point support this point of view.

When a dust-free liquid is irradiated by a beam of monochromatic radiation and the light scattered by it is analysed by means of an ordinary spectrograph, a large part of the scattering which owes its origin to the fluctuations of optical density to have unaltered frequency. Examination of this " unmodified " scattering with the aid of a high-resolving power interferometer such as the Fabry-Perot Etalon however reveals it as split into three components one in the position of the original line and the other two appear displaced from it to the positions predicted by Brillouin's theory of light scattering (1922). The outer or Brillouin components owe their origin to pulsations of optical density taking place over an extended region in the fluid and travelling through the medium with the velocity of sound waves and thus giving rise to a Doppler effect. The appearance of a central component is attributed partly to the 'Q' branch of the orientation scattering and partly to the fluctuations of optical density of a quasi-static or static character. It is clear from the facts that an interferometric study of light scattering enables us to make a frequency analysis of the fluctuations of

optical density and to ascertain whether they occur with or without phase relationship within the fluid.

Such an investigation has not been carried out so far in the case of liquid mixtures and accordingly an interferometric study of the light scattered by liquid mixtures of both miscible and immiscible types was undertaken and the results are described in the present communication.

2. *Experimental Details*

The critical mixture chosen for the investigation consisted of (1) a polar and a non-polar liquid, *viz.*, methyl alcohol and normal hexane and (2) two polar liquids, *viz.*, *iso*-butyric acid and water. Two ordinary mixtures were also chosen. One was of two non-polar liquids namely benzene and *n*-heptane and the other of two polar liquids, namely ethyl alcohol and water which are miscible at all temperatures. Great care was taken to prepare all these mixtures perfectly dust-free and in the case of the critical mixtures with the correct critical composition, since the critical opalescence is most pronounced in such mixtures. The procedure adopted was as follows:—30% by weight of freshly distilled methyl alcohol was mixed with 70% by weight of distilled *n*-hexane and the mixture transferred into a flask having a capacity of about 400 c.c. This flask was connected to a Wood's tube of nearly 300 c.c. capacity and having a diameter of 3 cm. and length 20 cm. The flask containing the mixture was immersed in ice and the system evacuated and sealed off. The mixture was then distilled two or three times into the Wood's tube, each time washing the distillate back into the flask, the final distillation being carried out slowly till the whole of the mixture was almost completely distilled into the experimental tube. The same procedure was adopted for the *iso*-butyric acid-water mixture, the critical composition in this case being 50% by weight of each of the components. The two ordinary mixtures were also prepared in the same manner, their composition being 50% by volume of each of the components.

3. *General Statement of Results*

1. *Methyl Alcohol* n-*Hexane Mixture.*—The interference pattern obtained with a Fabry-Perot Etalon having 5 mm. separation for the 4810 A.U. radiation of the zinc-mercury amalgam arc, for this mixture at 30° C. is reproduced in Fig. (2) (*d*) [(the critical temperature being 29° C.). along with those of methyl alcohol and *n*-hexane (see Fig. (2) (*b*) and (*c*)]. Fig. 1 (*a*, *b* and *c*) gives the microphotometer curves for the same radiation. Both methyl alcohol and *n*-hexane have very intense and well-defined

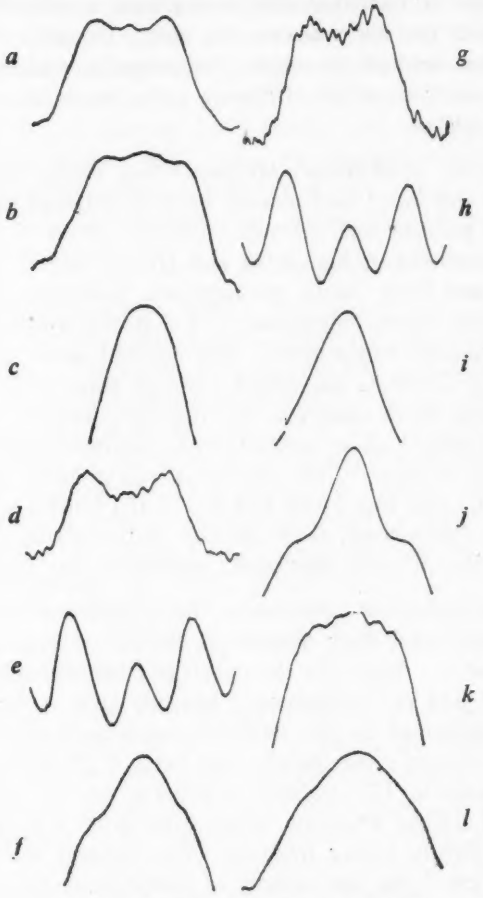

FIG. 1. Microphotometric Curves for Liquids

(*a*) Methyl alcohol
(*b*) *n*-Hexane
(*c*) Methyl alcohol–*n*-Hexane mixture
(*d*) Ethyl alcohol
(*e*) Water
(*f*) Ethyl alcohol-Water mixture
(*g*) *iso*-Butyric acid
(*h*) Water
(*i*) *iso*-Butyric acid–Water mixture
(*j*) Benzene
(*k*) *n*-Heptane
(*l*) Benzene–*n*-Heptane mixture

Brillouin components, their wave number shift $\delta\nu$ respectively being 0·197 cm.$^{-1}$ and 0·21 cm.$^{-1}$. The velocity of sound waves in these liquids is 1068 m./sec. for methyl alcohol and 1096 m/sec. for *n*-hexane. The

central component for methyl alcohol is very weak compared to the Brillouin components, while that for *n*-hexane has nearly the same intensity. In the mixture, however, we find the central component appearing with enormous intensity, but no trace of the Brillouin components is detected even in highly exposed plates.

2. *Iso-Butyric Acid-Water Mixture.*—The Fabry-Perot patterns for *iso*-butyric acid and water have already been investigated by Venkateswaran (1942) and his pictures and microphotometric curves for the same are reproduced respectively in Fig. 2 (*k*) and (*l*) and Fig. 1 (*g*) and (*h*). As can be easily seen from these photographs *iso*-butyric acid shows only an extremely weak central component and a strong continuum between the two intense Brillouin components. The spectral shift of the Brillouin components is 0·22 cm.$^{-1}$, the sound velocity being 1125 m./sec. Water on the other hand shows clearly all the three components the central component being slightly weak in comparison to the outer ones. The Brillouin shift for water is 0·28 cm.$^{-1}$, the velocity of sound being 1509 m./sec. The mixture at 30° C. [see Fig. 2 (*m*) and Fig. 1 (*i*)] however, shows no trace of the Brillouin components, as in the case of the mixture discussed above, in spite of the fact that the interference pattern is very intense.

3. *Benzene-n-Heptane Mixture.*—The interferometric patterns of benzene *n*-heptane and their mixture at 30° C. are respectively given in Fig. 2 (*p*), (*q*) and (*r*). Fig. 1 (*j*), (*k*) and (*l*) give the microphotometric curves for this mixture and its components. Benzene gives a very strong central component accompanied by two Brillouin components on either side of it, and of half its intensity; the spectral shift being 0·27 cm.$^{-1}$ and the velocity of sound propagation 1275 m./sec.; *n*-heptane on the other hand, gives two strong and diffuse Brillouin components with a fairly sharp central component of slightly higher intensity. The spectral shift of these components is 0·23 cm.$^{-1}$ and the velocity of sound propagation is 1161 m./sec. The mixture also unlike the above two critical mixtures shows all the three components. The mixture pattern has roughly the appearance of being a superposition of the patterns of the two component liquids. The velocity of sound propagation in this mixture is 1240 m./sec.

4. *Ethyl Alcohol-Water Mixture.*—The Fabry-Perot patterns obtained for the component liquids, *viz.*, ethyl alcohol and water by Venkateswaran (1942) are reproduced in Fig. 2 (*g*) and (*h*) along with that of the mixture Fig. 2 (*i*) obtained in the present investigation. Fig. 1 (*d*), (*e*) and (*f*) gives the microphotometric curves for the same. Here, as in the case of *iso*-butyric acid, ethyl alcohol gives slightly diffuse and very intense Brillouin components

accompanied by a sharp weak central component. As has been already mentioned in an earlier paragraph, water shows all the three components, the central component being slightly weaker than the outer ones. The mixture, on the other hand, shows a striking change. The central component exhibits a marked increase in intensity, the Brillouin components becoming relatively weaker than those of the component liquids. The velocity of sound propagation in water and ethyl alcohol is respectively 1509 m./sec. and 1130 m./sec. while that in the mixture is 1480 m./sec.

4. *The Effect of Temperature*

The two critical mixtures methyl alcohol-*n*-hexane and *iso*-butyric acid-water were also investigated at temperatures higher than the critical solution temperature. The interference patterns for methyl alcohol-*n*-hexane mixture at 40° C. and 50° C. are reproduced in Fig. 2 (*e*) and (*f*) and that of *iso*-butyric acid-water at 90° C. and 120° C. in Fig. 2 (*n*) and (*o*). The former shows no trace of the Brillouin components in spite of the fact that the mixture has been raised 21° C. above the critical temperature. The central component, however, becomes relatively sharper and weaker at higher temperatures. In *iso*-butyric acid-water mixture, on the other hand, the Brillouin components are clearly, though weakly seen at 90° C. The central component continues to be intense at all temperatures. The relative intensity of the shifted components to the central component becomes greater at 120° C.

Another fact of observation worthy to be recorded here is the relative intensity of the 5461 and 4358 A.U. radiations in the scattered spectrum. At the critical temperature, the green radiations are nearly as intense as the 4358 A.U. radiations; but as the temperature is raised by 20° C. or more, they become extremely weak, suggesting that the intensity of the opalescent light at the critical condition is proportional to λ^{-n} where n has a value much less than four [Andant (1924) and Rousset (1934)] and that at higher temperatures it tends to become proportional to λ^{-4}.

5. *Discussion of the Results*

The appearance of the Brillouin components in ordinary mixtures and the absence of the same in critical composition mixtures is very significant. One factor which might hinder the appearance of the Brillouin components is the abnormal increase in the coefficient of viscosity at the critical solution temperature reported by several authors such as Ostwald and Malss (1933) and Zofia Szafranska (1935). The effect of viscosity on the fine-structure components exhibited by pure liquids has been clearly brought out by

the studies of Venkateswaran (1942). Two important facts emerge out of his study on highly viscous liquids, *viz.*, (1) that the Brillouin components are present though feebly in the case of liquids like glycerine and castor oil, even at room temperature, (2) that the ratio of the intensity of the Brillouin components to the central component increases rapidly with rise of temperature. In the case of glycerine at 110° C. the intensity of the Brillouin components is comparable to that of the central component.

Similar temperature investigations on methyl alcohol-*n*-hexane and *iso*-butyric acid-water mixture ought to reveal marked changes in the relative intensity of the Brillouin components to the central component. On the contrary, the present investigations show no trace of the Brillouin components in the case of methyl alcohol-*n*-hexane mixture even when its temperature is raised by 20° C. above its critical temperature and for *iso*-butyric acid by 45° C. In the latter, the Brillouin components appear weakly at 90° C. and when the temperature is raised to 120° C. they brighten up, but not to the extent expected for a range of 30° C. These results therefore suggest that changes of viscosity as such cannot account for all the observed facts.

We are therefore obliged to interpret the absence of Brillouin components as indicating that the critical mixtures are composed of molecular clusters of size comparable to the wave-length of the sound waves and which are randomly distributed thus preventing the free propagation of sound waves in these mixtures. The results of the study of the effect of temperature on the light-scattering by these two mixtures indicate that they remain as emulsoids for a wide range of temperature above their critical points about 20° C. for methyl alcohol-*n*-hexane mixture and 45° C. for *iso*-butyric acid-water mixture. This conclusion is in agreement with the observations of Krishnan (1935) who finds that the range over which the clusters are detectable in the case of critical composition mixtures is more than 30° C.

On the other hand the appearance of the Brillouin components with intensity comparable to that of the pure liquids in ordinary mixtures indicates that there is no likelihood of the existence of large molecular clusters in them. X-ray and other optical evidences lend support to this view. The enhanced intensity of the central component however suggests that the composition scattering forms an important part of the total scattering in these mixtures as well.

In conclusion, the author desires to express her gretaful thanks to Prof. Sir C. V. Raman for his continued interest and encouragement in the course of the work. My thanks are also due to Dr. C. S. Venkateswaran for helpful suggestions.

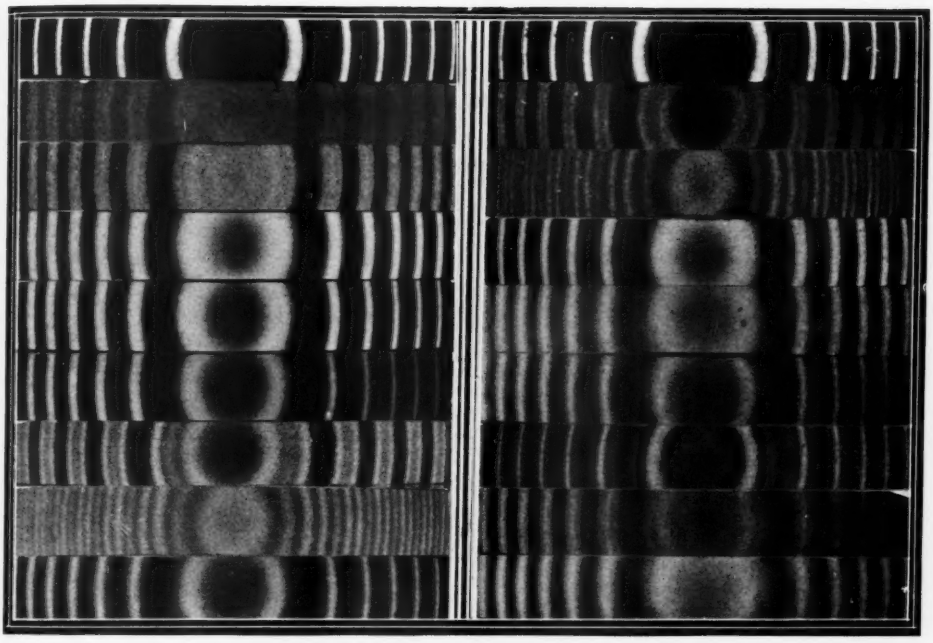

FIG. 2. Interferometric Patterns of Light Scattering in Liquids

(*a*) Direct Zn line (4810)
(*b*) Methyl alcohol (30° C.)
(*c*) *n*-Hexane (30° C.)
(*d*) Methyl alcohol–*n*-Hexane mixture (30° C.)
(*e*) Do. (40° C.)
(*f*) Do. (50° C.)
(*g*) Ethyl alcohol (30° C.)
(*h*) Water (30° C.) (4078 Hg)
(*i*) Ethyl alcohol–Water mixture (30° C.)
(*j*) Direct Zn line (4810)
(*k*) *iso*-Butyric acid (30° C.)
(*l*) Water (30° C.) (4078 Hg)
(*m*) *iso*-Butyric acid–Water mixture (30° C.)
(*n*) Do. (90° C.)
(*o*) Do. (120° C.)
(*p*) Benzene (30° C.)
(*q*) *n*-Heptane (30° C.)
(*r*) Benzene–*n*-Heptane mixture (30° C.)

6. *Summary*

The fine structure of the opalescent light of two critical composition mixtures, *viz.*, methyl alcohol-*n*-hexane and *iso*-butyric acid-water, has been studied at the critical solution temperature and higher temperatures. At the critical solution temperatures the Brillouin components are absent in either cases, while the central component is very intense. The former do not appear in the alcohol-hexane mixture even when its temperature was raised by 20° C. In the acid-water mixture, the shifted components are weakly present at 90° C. and found to increase in intensity at 120° C. In mixtures of miscible liquids namely benzene-*n*-heptane and ethyl-alcohol water also included in the present study, the Brillouin components are present prominently. The central component, however, shows enhanced intensity. These results indicate that a critical mixture at or above the critical solution temperature consists of molecular aggregates whose size is comparable to the wave-length of light and hence does not permit of the propagation of ordered sound wave trains of thermal origin. The significance of these results with reference to the Krishnan effect exhibited by binary liquid mixtures and the X-ray and other evidence is discussed.

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ACTIVATION OF NITROGEN IN THE PRESENCE OF MERCURY

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§1. Appreciable divergence of opinion exists in regard to the effect of mercury on the life of active nitrogen. Strutt,[1] now Lord Rayleigh, observed: "Mercury gave an explosive compound, when its vapour was allowed to mix with active nitrogen." This was traced to the formation of mercury nitride. In a later paper[2] Rayleigh remarked: "No apparent effect is produced when the active gas is passed over clear cold metals or over a film of mercury held on copper; ....if a small quantity of mercury is placed in the bottom of a fairly wide tube, and shaken while the active gas passes over it, all luminosity is extinguished and the mercury becomes foul.....If the shaking is discontinued, the glow again passes." It must be emphasised that this role of mercury in the above-mentioned and similar experiments of Rayleigh and others is fundamentally different from the finding that on extreme purification nitrogen fails to give the afterglow and that the introduction of but a small proportion of a foreign substance, *e.g.*, mercury vapour, restores it.[2] To quote Rayleigh[2]: "A drop of mercury was placed inside a tube so that by warming, it could be made to give off vapour, which mingled with the nitrogen stream, a marked restoration of the glow was observed, though it was less brilliant than that obtained with the best catalysts." On the other hand, Kichelu and Basu[3] found: "For the measurement of pressure up to a certain stage, say 0·05 mm. Hg, a McLeod gauge was conveniently employed; but as the pressure of nitrogen became smaller any traces of mercury vapour had very destructive effect upon the life of active nitrogen, which quickly spent itself by acting upon mercury..... In the absence of liquid air it was found impossible to stop a slight diffusion of mercury vapour from the pump..... Even after these precautions, we did not find it possible to work with nitrogen below 0·02 mm." J. K. Robertson[4] also observed that "the addition of mercury vapour at once destroys the glow." As a manometric liquid and for working the evacuating pumps, mercury is almost indispensable in any work on gas reactions. In view of this and especially the above marked differences of observation in the literature, it was of interest to investigate the action of active nitrogen on mercury over a wide range of conditions.

§2. Fig. 1 shows the essential parts of the apparatus employed. The cylindernitrogen containing 0·78% oxygen was passed through a train of bulbs filled with alkaline pyrogallol to remove oxygen; it was then stored for about 3 days before use in an aspirator in which was hung a muslin bag containing some considerable quantity of freshly cut yellow phosphorus.

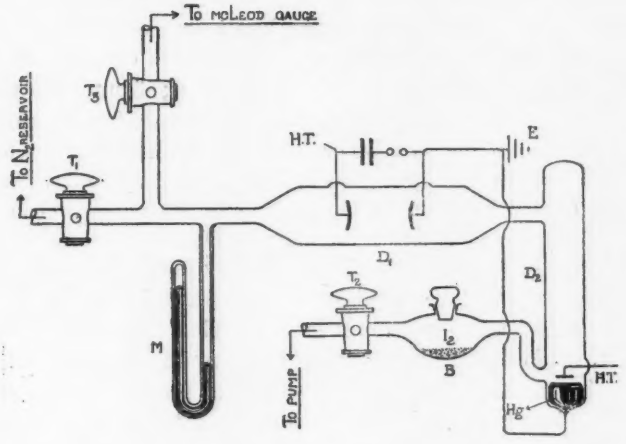

Fig. 1

No material differences were, however, observed so far as the nature of the results now to be reported are concerned, whether the gas was used after this purification or directly from the cylinder. By operating the suction pump and control of the stop-cocks the requisite pressure of nitrogen shown by the mercury manometer M was obtained; very low pressures were observed with a McLeod (not shown in the Fig.) connected at T_3. The gas was activated by a condensed, spark-in-series discharge in the Crookes' type tube D_1 fitted with well-cleaned aluminium electrodes about 1 cm. in diameter and 1·5 cm. apart; the electrode on the suction side was earthed. One more discharge tube D_2 disposed vertically and in series with D_1 contained some pure mercury at the bottom, which was earthed; the other electrode was connected to the H.T. as in D_1 when required. D_2 was immersed in an oil-bath whose temperature could be varied in the range 30–300° C. A small amount of powdered iodine in B served as a sensitive detector of active nitrogen on account of the very characteristic luminescence which it excites when brought into contact with iodine. This served to detect active nitrogen which was too feeble for visual observation. The various parts of the apparatus

between T_1 and T_2 were connected by fused glass-joints and the system was repeatedly tested for vacuum on a Töpler, before final observations.

§3. In the first series of experiments nitrogen streamed at a pressure of 2–3 cm. Hg was activated in D_1. The yellowish after-glow was well noticeable in D_2 on switching off the exciting voltage; with this on, and a well regulated suction, the glow was observable from D_2 upto T_2. It was just tinged with the characteristic green luminescence of mercury when the gas was flowing through D_2, which was kept at the ordinary temperature; was predominantly bluish-white due to iodine, in B; from T_2 to the exhaust end, the appearance was once more that of the normal after-glow of nitrogen. As the temperature of the bath was raised, the luminescence due to mercury predominated over the nitrogen glow in D_2. Above 140° C., mercury in D_2 boiled; this caused such an intensification of its luminescence that the after-glow of nitrogen was not perceptible in D_2. It is considered that this marked increase in the intensity of the mercury luminescence is appreciably due to transfer of energy by collisions of the second type, between part of active nitrogen and mercury vapour in D_2.[6] That the glow of active nitrogen was but masked or rather overshadowed by the mercury luminescence was shown by the fact that the streaming gas produced the characteristic emission of iodine in B and that the familiar after-glow of nitrogen was distinctly perceptible beyond B, *i.e.*, between T_2 and the exhaust.

§4. Two series of experiments were next made in which nitrogen was activated by exciting the discharge tube D_2 containing mercury as the earthed electrode: with D_2 at 28–30° C., the iodine detector B and the apparatus beyond it showed a well-marked after-glow over a wide pressure range of the streaming gas. The same results were obtained in which instead of streaming, the apparatus between T_1 and T_2 was filled with nitrogen at a pressure varied in the range 1–2 cm. Hg, and D_2 was excited intermittently. After each cessation of the discharge, an after-glow marked enough to be identified with a direct vision hand-spectroscope, was seen in D_2 and extending appreciably on either side of it, for a time which increased on decreasing the gas pressure. With the above arrangement *i.e.*, with static nitrogen, on reducing the gas pressure the duration of the after-glow increased progressively; it was 5–6 minutes at about 0·0018 cm. Hg.

In the second series of experiments, the bath surrounding D_2 was heated to 160° C., which produced a copious vaporisation of mercury. Nitrogen was then streamed at pressures varied from a few mm. to 2 cm. Hg. An *intense* after-glow was observed beyond D_2 upto T_2, which was in marked contrast when the activated gas was allowed to flow into neutral mercury

vapour.[2,3] It may be added that mercury in D_2 was fouled and showed the formation of mercury nitride when tested in the usual way.

There is considerable evidence to show the formation, under electrical discharge in mercury vapour, of the excited and meta-stable states; their proportion would increase with temperature. It is to be anticipated therefore, that subjecting nitrogen to an electrical discharge under such conditions would favour, as is actually observed, the intensification of the after-glow by (i) the diminution of the probability of collisional de-activation and (ii) the direct activation of the gas by energy transfer from the excited and metastable atoms of mercury,[6] and in collisions with the mercury ions moving under the action of the applied field.[7]

Summary

That active nitrogen is destroyed by contact with mercury is untenable as a general proposition. Their interaction produces the nitride and a variety of consequences depending upon the nature of conditions. When streamed into neutral mercury vapour, partial de-activation of active nitrogen and an excitation of the luminescence of mercury are the chief changes; the intensity of the latter increases with temperature and tends to overshadow that of nitrogen. When, however, the gas is excited with heated mercury as electrodes, the after-glow is markedly intense, which has been attributed to collisions with the excited and the metastable atoms of mercury, and its ions in the discharge space.

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SOME ASPECTS OF THE BIOGENESIS OF ANTHOXANTHINS

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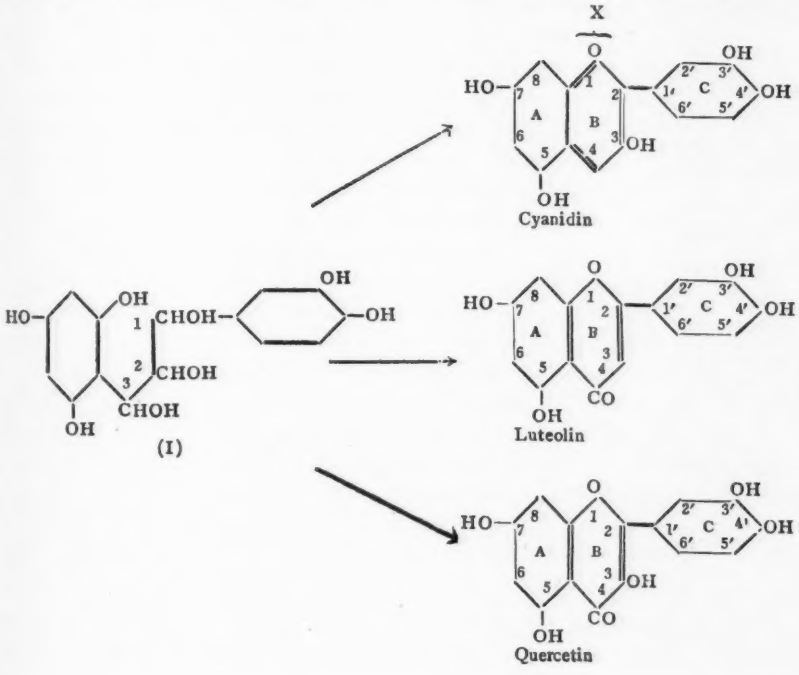
In a recent publication was given a brief account of our knowledge about naturally occurring flavones and flavonols whose constitutions have so far been definitely known.[1] They were arranged into different groups depending upon the number and the orientation of the hydroxyl groups in the benzopyrone part of the molecules. Their occurrence and their characteristic properties were also recorded. From the data thus presented it seemed to be possible to obtain information regarding the manner of their evolution in the plant kingdom. Possibility of success in this venture appeared to be greater particularly in view of the valuable conclusions which Robinson and his collaborators have recently arrived at in regard to the biogenesis of the anthocyanins.[2]

Anthocyanins and anthoxanthins are two groups of closely related sap soluble pigments. They are built upon the same pattern having 15 carbon atoms and 3 rings. Even before their chemical constitutions were definitely known, biologists were impressed by their association and they considered that anthocyanins were generated from the anthoxanthins by a process of oxidation taking place in the plant.[3] In support of this idea they could mention the comparatively later appearance of the bright red and blue colours due to anthocyanins and under conditions favourable to oxidation. When, as a result of the important work of Kostanecki, Perkin and others on the anthoxanthins and of Willstätter and his co-workers on anthocyanins, the chemistry of these two groups was made clear, the close chemical relation between them was established, but the anthocyanins represented a lower state of oxidation. Consequently the suggestion was made that anthocyanins were really products of reduction of flavones and flavonols, and no oxidation was involved.[3] On either of these two ideas which mean sequential evolution of the pigments, a correlation could be expected between the individuals of the different groups occurring in the same parts of the plants. For example, pairs like pelargonidin and kæmpferol, cyanidin and quercetin, delphinidin and myricetin, should occur. In the cases examined in this connection this correlation could not be found. Further, the presence

of pelargonidin with myricetin, delphinidin with kæmpferol was not uncommon. In the few cases where correlation was found, cyanidin and quercetin were involved and this could be considered as incidental to their prolific occurrence in the plant kingdom.

The difficulties were satisfactorily explained by Robinson who developed the idea of parallel origin of these groups of compounds from a common source[2]; different degrees of oxidation are involved in the production of anthocyanins, flavones and flavonols. According to his scheme no correlation is required between the members of the different groups occurring together. On the supposition that anthocyanins and other related substances are built up from simple carbohydrates through a series of aldol condensations, the hypothetical intermediate (I) was suggested as the basis from which all these compounds are derived. The intermediate can be constructed from two hexose units and one triose, and is in the same state of oxidation as a carbohydrate. The central three-carbon fragment of (I) may be modified in several ways to give different end products. For example, oxidation at C_1 leads to the formation of cyanidin, at C_3 to the flavone, luteolin and at both C_2 and C_3 or at C_1 and C_3 to the formation of the flavonol quercetin.



During the past several years Robinson and his collaborators have been engaged in a survey of anthocyanins occurring in nature. Making use of a quick method of analysis requiring small quantities of plant material they have been able to study a very large number of samples and thus provide sufficient material for statistical analysis. It has been found by them that the basic structures concerned are remarkably small in number being pelargonidin, cyanidin and delphinidin. Only a few rare cases correspond to flavones, *i.e.*, they lack in a hydroxyl group in the 3 position; gesnerin and carajurin are the rare examples. Further from their data it could be concluded that cyanidin is the primary member of the anthocyanidins and the production of delphinidin (oxidation) and pelargonidin (reduction) involve one stage more in evolution. These are remarkable results and give great support in favour of structure (I) as the common precursor.

The study of the biogenesis of anthoxanthins is more difficult for several reasons. In contrast to the few basic types found in the anthocyanins the variations in flavones and flavonols are quite numerous. There are at least 18 flavonols and 12 flavones of established constitutions. Quick methods of analysis of plant materials for anthoxanthins have not yet been developed and hence it has not been possible to analyse such large number of samples as for anthocyanins. Further, whereas anthocyanins usually occur single and in some cases with one more as minor component, anthoxanthin mixtures are more frequently complex. Consequently the number of cases of complete analysis are limited and deductions cannot therefore be comprehensive. However, as stated at the beginning it seems to be possible to arrive at some conclusions based on the following considerations :—(1) since anthocyanins and anthoxanthins are closely related, the results obtained from the more comprehensive study of the former could be accepted as applicable for the latter also, (2) the association of compounds in the same part of a plant, in different parts of the same plant and in closely related plants could be utilised as evidence of biogenetic relationship. In regard to alkaloids and other well-defined groups of naturally occurring compounds it is quite well known that plants of the same family contain the same compounds or closely related compounds. The differences amongst them are only in finer details and not in fundamentals. The minor changes mainly due to oxidation, reduction and dehydration could be brought about differently or to different stages in different members of a group of plants, in different parts of the same plant or even in the subdivisions of the parts. But the main framework may be said to be evolved in the same way.

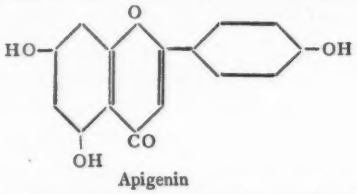
From consideration (1) given above, it follows that quercetin should be considered to be the simplest of the flavonols and luteolin the simplest of the

flavones and that the others require more stages for their evolution. This is also supported by the abundance of these two representatives in nature. Quercetin is the most frequently encountered anthoxanthin. Gisvold and Rogers[4] give figures showing that out of 268 cases where anthoxanthins have been identified, 220 have the 3′: 4′-dihydroxy orientation in the substituted phenyl group. It could further be stated in general terms that as in the case of the anthocyanins, here also variations in the number of hydroxyl groups in the side phenyl nucleus is possible by the introduction of one stage more of oxidation or reduction, the positions 3′ and 5′ being involved. Support for the above statement is available from the association of compounds as they occur in nature and it is given later in this paper. The state of complete reduction leaving no hydroxyl in the side phenyl nucleus is common in the anthoxanthins and is a distinctive feature. But a more prominent characteristic of these compounds which does not find a parallel in the anthocyanins is the large variation in the state of oxidation of the benzene ring (A). In the latter group of pigments, two hydroxyls are invariably present in the 5: 7-positions. Resoanthocyanins have not been found in nature. Carajurin represents an exceptionally rare case; but its occurrence indicates that the possibility of modification of ring (A) even in the anthocyanins cannot be excluded, though it may be uncommon. But in the anthoxanthins the variation in the number of hydroxyls is from 0 to 4. An explanation of this could probably be found in the marked difference in reactivity of the nuclear positions of anthoxanthins and anthocyanins after the structures have been formed from the common precursor, the modifications not taking place earlier. If quercetin and luteolin should be the simplest of the flavonols and flavones (containing hydroxyls in 5: 7 positions) the biogenesis of the others should involve further stages of oxidation and reduction. How far this conclusion is justified and in what manner the variations arise can be indicated in a general way by a study of the pigment associations made below. Though in most of the cases considered the pigments occur as glycosides, only the aglycones are mentioned and used in the discussion. The nature of the sugars involved and the positions they occupy do not seem to be relevant for the present purpose. Similar considerations are applicable to the methyl ethers also. Further the list is not claimed to be comprehensive; typical cases alone are taken up and attention is particularly focussed on the evolution of special structures found only in the anthoxanthins and not in the anthocyanins.

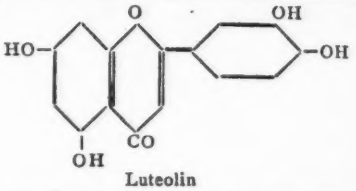
I. Some associations of flavones and flavonols in which the side phenyl nucleus has different number of hydroxyl groups are given below. It could be noticed that compounds with one hydroxyl (4′-position) or three

hydroxyls (3′, 4′ and 5′) are accompanied by those with two hydroxyls (3′ and 4′), indicating the evolution of the first two from the third. There seems to exist no definite cases where types with one and three hydroxyls in the side phenyl nucleus occur together, particularly to the exclusion of the 3′:4′ dihydroxy compounds.

(*i*) The combination of apigenin and luteolin is frequently met with in different parts of the same plant or in plants of allied species and varieties.



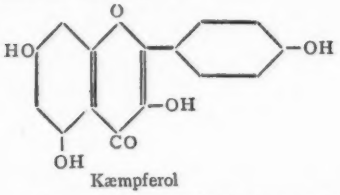
Apigenin



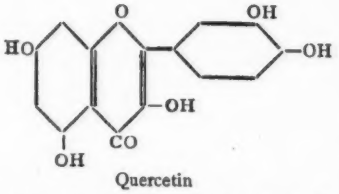
Luteolin

Apigenin occurs in the seeds of parsley, while in the stem and the leaves luteolin (as its monomethyl ether) is also present along with it.[5] Both of them are found in the yellow variety of *Antirrhinum majus*[6] and also in *Reseda luteola* (Weld.).[7] The two varieties of *Chrysanthemum* yield the two related pigments; *C. indicum* contains luteolin and *C. leucanthemum* apigenin.[8]

(*ii*) Kæmpferol is known to be found along with quercetin or its methyl ethers in several sources.



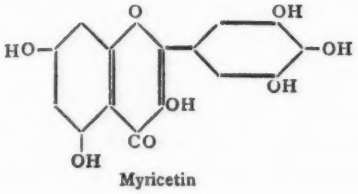
Kæmpferol



Quercetin

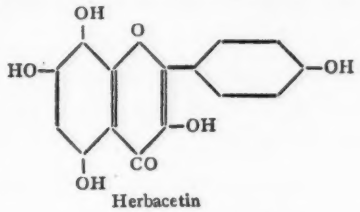
The stems of *Equisetum arvense* contain both these pigments, besides luteolin.[10] In senna leaves kæmpferol and isorhamnetin (3′-methyl quercetin) are present.[9] The same combination occurs in the flowers of *Delphinium Zalil* (Asbarg).[11] Kæmpferol and isorhamnetin along with kæmpferol monomethyl ether are found in *Rhamnus catharticus*.[9]

(*iii*) Quercetin and myricetin are both present in *Myrica nagi* and *Coriaria myrtifolia*.[9]

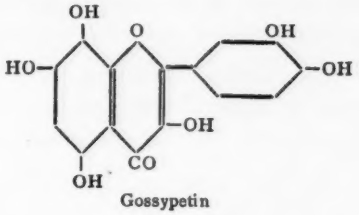


Myricetin

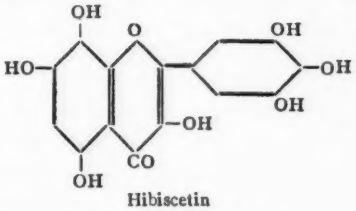
(*iv*) Indian cotton flowers (*Gossypium herbaceum* and *indicum*) contain gossypetin and herbacetin,[12] and in *Hibiscus sabdariffa* gossypetin and hibiscetin occur, the latter being the major component.[13]



Herbacetin

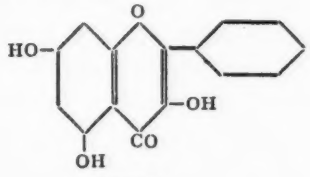


Gossypetin

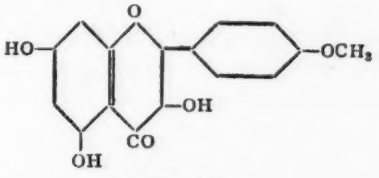


Hibiscetin

(*v*) Galangin, its monomethyl ether and kæmpferide occur together in the galanga root.[9] This example could be taken as indicating the sequence from the di- and monohydroxy side-phenyl nucleus to one devoid of hydroxyl groups.



Galangin



Kæmpferide

II. In some plants quercetin occurs along with other compounds which contain one more hydroxyl group in ring (A).

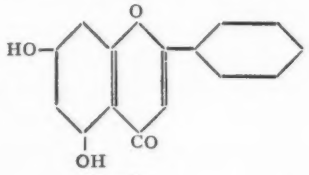
(*i*) The American cotton flowers, *G. hirsutum* contain as the sole component quercetin,[14] whereas the Indian varieties *G. herbaceum* and *G. indicum* contain gossypetin and herbacetin besides quercetin, gossypetin being the major component.[12]

(*ii*) The flowers of two species of *Thespasia* have so far been examined. *T. lampas* was studied by Perkin and was found to contain quercetin.[15] *T. populnea* has been shown by Rao and Reddy[16] to contain herbacetin besides populnetin (a flavone whose constitution is not yet settled[16a]).

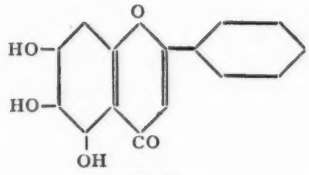
From the above data, it seems to be reasonable to infer that quercetin is the primary stage, and gossypetin indicates a higher stage of evolution involving oxidation which leads to the formation of the hydroxyl group in position 8. Herbacetin is a subsequent stage of reduction resulting in the disappearance of the hydroxyl group in 3′-position in ring (C).

III. 5:6:7 Combination of three hydroxyl groups in ring (A) occurs fairly frequently. This also seems to be an evolution from the more fundamental 5:7-dihydroxy types involving oxidation.

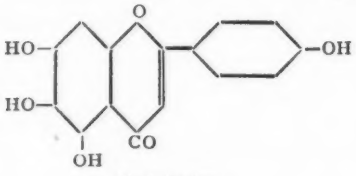
(*i*) The stem and root barks of ***Oroxylum indicum*** contain baicalein and its 6-methyl ether, oroxylin-A. Besides these two, the stem bark contains chrysin.[17] Thus the relation between the 5:7-dihydroxy and the 5:6:7-trihydroxy compounds is indicated.



Chrysin



Baicalein

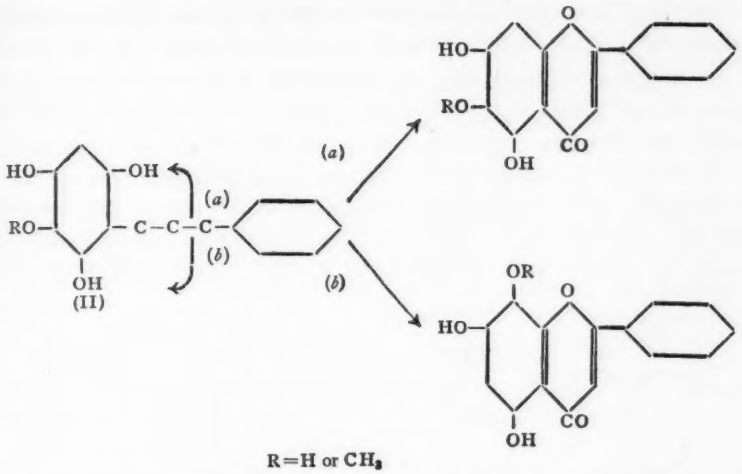


Scutellarien

(*ii*) Regarding the existence of stages in the reduction of ring (C) the composition of the parts of different species of ***Scutellaria*** is useful. The leaves and flowers of ***S. baicalensis*** and ***S. altissima*** contain scutellarein[18] whereas the roots of ***S. baicalensis*** contain baicalein.[19]

(*iii*) Another interesting point that should be noted here is that in the roots of ***S. baicalensis***, wogonin, the 5:7:8 analogue of oroxylin-A is also found to be present[19] and the ether group is in the 8-position. There are two ways of explaining this phenomenon:

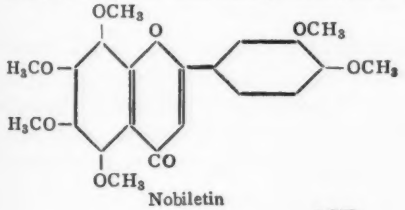
(*a*) the 5:6:7 and the 5:7:8 combinations arise independently from the 5:7-dihydroxy compound by oxidation affecting position 6 or position 8 independently; (*b*) the oxidation takes place at some stage prior to the closure of the pyrone ring and the ring closure takes place in two different ways, one leading to the 5:6:7 and the other to the 5:7:8-configuration:



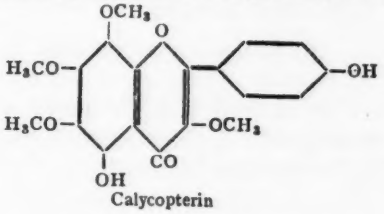
R=H or CH_3

The existence of the second possibility is indicated by some laboratory experiments. But they are not numerous enough to be conclusive. The association of 5:6:7 and 5:7:8 types is found so far in one case only. It may not be, therefore, possible to say definitely which of the above two alternatives is more plausible. The first is probably more simple and also avoids certain other difficulties. It is, however, more definite that they are both derived from the 5:7-dihydroxy precursor.

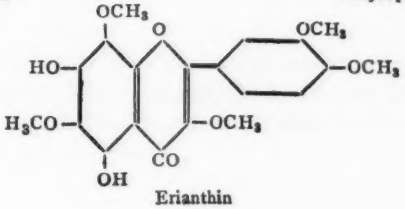
IV. So far cases of one stage oxidation of the benzene ring (A) have been discussed. The idea could be extended to complete oxidation resulting in tetrahydroxy benzopyrone systems. Several of this type have been recently discovered. It is noteworthy that all these, nobiletin, calycopterin and erianthin are partially or completely methylated presumably in order to escape oxidation.



Nobiletin



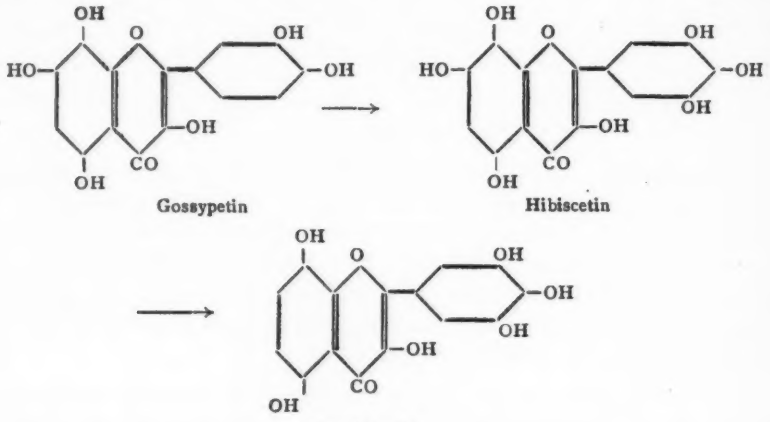
Calycopterin



Erianthin

V. The following associations in closely related species indicate the existence of a stage of reduction of the hydroxyl group in position 7.

(*i*) ***Hibiscus sabdariffa*** contains **hibiscetin** as the main component and gossypetin and sabdaretin as the minor portion,[13] whereas the closely related species, ***H. cannabinus*** contains cannabiscetin as the sole component.[20] The constitution of sabdaretin is not known yet and hence it is omitted from consideration. The relationship between the others can be expressed by the following formulæ and it seems to be reasonable to conclude that cannabiscetin is derived from hibiscetin by a stage involving reduction affecting position 7.



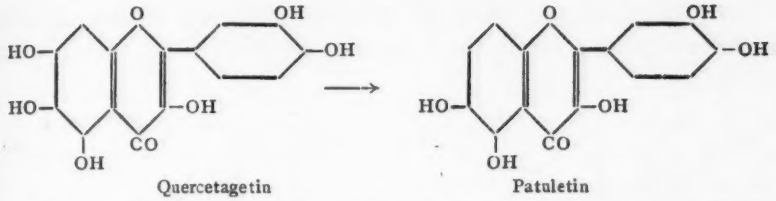
Gossypetin Hibiscetin

Cannabiscetin

It may be mentioned here that in one experiment a small amount of cannabiscetin was found in ***Hibiscus sabdariffa***; but it could not be isolated from other samples.

(*ii*) In this connection may be considered the components of the species of ***Primula***. Primetin, 5:8-dihydroxy flavone, is obtained from the leaves of ***P. modesta***.[21] It is known that the flowers of ***P. sinensis*** contain pelargonidin or delphinidin glycosides depending on the variety.[22] Obviously the compounds containing the 5:7 combination of hydroxyls are common in these plants and the evolution of the 5:8 type through the intermediate 5:7:8 is possible.

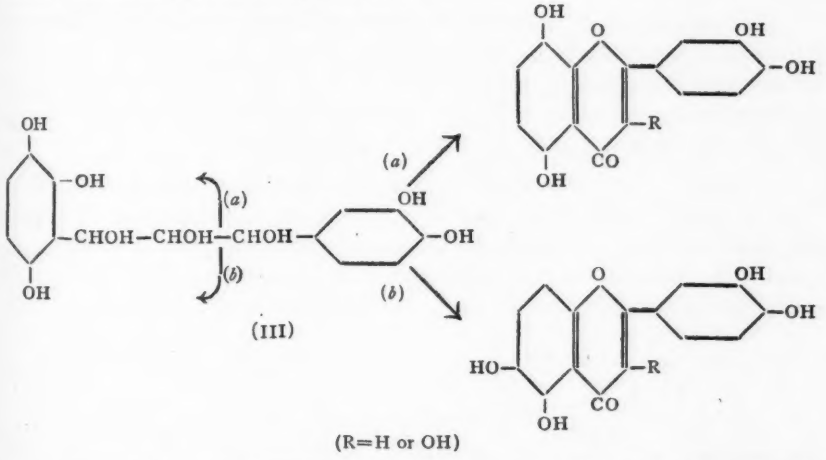
(*iii*) A very similar case arises in the two closely related flowers of the compositæ, ***Tagetes erecta*** and ***T. patula***. The former contains quercetagetin and the latter patuletin in which the hydroxyl in the 7-position is missing.[23]



Quercetagetin Patuletin

The occurrence of the above-mentioned 5:8 and 5:6 combinations of hydroxyl groups which are not so common seems to be due to a stage involving reduction of 5 : 7 : 8 or 5 : 6 : 7 combinations whereby the 7-hydroxyl is lost. This process is obviously very rare but seems to exist definitely.

It could be suggested that the 5:6- and the 5:8-dihydroxy compounds are evolved from hydroxy quinol as shown below (III) instead of phloroglucinol present as part of the precursor (I).



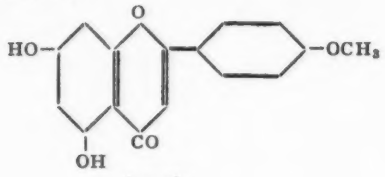
(III)

(R=H or OH)

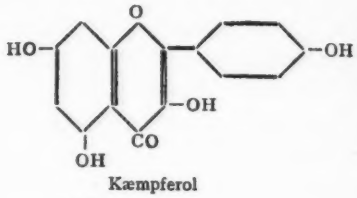
But this idea is neither supported by the considerations of associations and plant relations discussed above nor by the occurrence of hydroxyquinol nucleus in other groups of compounds found in nature nor by the reactivity of this nucleus.

VI. There are two cases of compounds, fisetin and robinetin in which there is no hydroxyl group in the 5-position, only one being left in the 7th position. Are they evolved quite independently of the fundamental 5:7 compounds or are they derived from them? The second alternative is supported by the following considerations:—

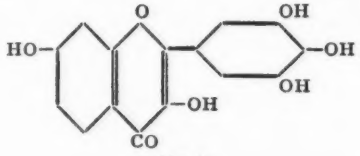
(*i*) The leaves and bark of ***Robinia pseudacacia*** contain acacetin,[24] the flowers contain kæmpferol,[25] whereas the wood (dye wood) contains robinetin.[26]



Acacetin



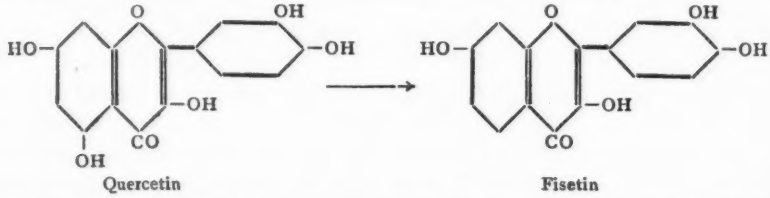
Kæmpferol



Robinetin

The association of 5:7 combinations with a compound containing only 7-hydroxyl group leads to the impression that it is derived from the fundamental dihydroxy compounds by a stage of reduction involving the hydroxyl in the 5-position. The possibility of oxidation or reduction in the side phenyl nucleus should be taken independently.

(*ii*) Other examples which support the above idea are obtained from "young fustic" and yellow cedar both of which are dye woods. The stem and branches of ***Rhus cotinus*** constituting "young fustic" contain fisetin, whereas the leaves of the plant (Venetian sumach) yield myricetin. In yellow cedar, fisetin is present in the stem and quercetin in the leaves.



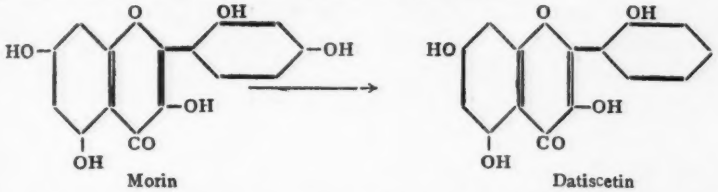
Quercetin Fisetin

It is not unreasonable to consider fisetin as representing a later stage involving reduction resulting in the disappearance of the 5-hydroxyl found in quercetin. Myricetin, of course, is related to quercetin in a different way.

(*iii*) As a further example of this type may be mentioned pratol (7-hydroxy-4′-methoxy flavone) isolated by Power and Salway from the flowers of ***Trifolium pratense***[27] and later by Rogerson from other species[28]; it occurs in these along with quercetin and isorhamnetin.

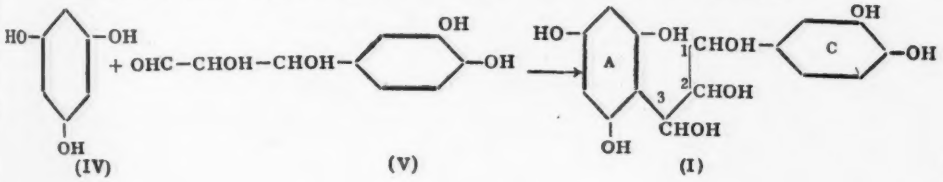
From the data presented above the following conclusions could be arrived at. (1) The theory of Robinson regarding the origin of anthocyanins and anthoxanthins from a common precursor is supported. (2) The conclusion of Robinson and his collaborators regarding the stage of oxidation of ring (C) in anthocyanins could be applied to anthoxanthins also, *i.e.*, 3′ : 4′ combination of hydroxyls is the simplest and 3′ and 3′ : 4′ : 5′ combinations involve more stages. In the anthoxanthins complete reduction of the hydroxyls in ring (C) is represented by a large number of examples. (3) Superimposed on the changes brought about in ring (C), ring (A) is quite easily susceptible to changes involving oxidation and reduction of nuclear positions. 5 : 7 Combination of hydroxyl groups seems to be the primary state; 5 : 6 : 7- and 5 : 7 : 8-trihydroxybenzene structures involve a stage of oxidation and 5 : 6 : 7 : 8-tetrahydroxybenzene nucleus results from a further stage of the same nature. 5 : 8-and 5 : 6-dihydroxy types seem to result from trihydroxy types involving a stage of reduction leading to the disappearance of the 7-hydroxyl. Similar reduction seems to produce fisetin and robinetin in which the hydroxyl in the 5-position is lacking.

There is one more point that requires consideration. It relates to the occurrence of the two rare cases of flavonols morin and datiscetin. The former is found in " old fustic " (wood of *Chlorophora tinctoria*) and in jak wood whereas the latter is present in the leaves and roots of the bastard hemp. In the roots datiscetin is accompanied by galangin.[29]



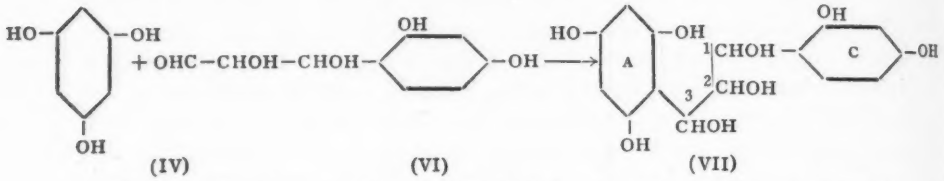
Morin Datiscetin

They have one hydroxyl group in the 2′-position. Obviously they belong to a rare type, but their occurrence may be taken as indicating the existence of a small variation from the fundamental precursor proposed by Robinson. This precursor is considered to be obtained from the union of two parts (IV) and (V), (IV) being phloroglucinol representing a hexose unit and (V) a nine carbon system derived from a hexose and a triose.



(IV) (V) (I)

If the possibility of a variation in part (V) could be conceded as shown in (VI), then an alternative precursor (VII) having a resorcinol structure in the side phenyl nucleus instead of the catechol structure becomes available.



(IV) (VI) (VII)

By the subsequent oxidation at C_1 and C_3 or at C_2 and C_3 morin could be obtained and from it involving a stage of reduction datiscetin could be evolved. But the occurrence of these types does not seem to be more common. The association of galangin with datiscetin is noteworthy. It could probably be derived from both catechol and resorcinol types of the side phenyl nucleus by complete reduction of the hydroxyl groups present in this part.

Summary

Based on the theory of Robinson *et al.* regarding the biogenesis of anthocyanins and on a detailed consideration of the occurrence of anthoxanthins (flavones and flavonols) in plants, a scheme of evolution of the various types of anthoxanthins is developed and discussed. Besides the existence of stages of oxidation and reduction of the side phenyl nucleus, similar modifications by oxidation and reduction of the benzopyrone part is involved. The possibility of a variation in ring (C) of the common precursor (I) as shown in structure (VII) leading to certain rare cases having hydroxyl groups in 2′- and 2′ : 4′-positions is suggested.

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ERRATUM

Vol. XVII, No. 6, Section A, June, 1943

Page 222, line 16: *For* $D_x^r [\alpha_\mu (x)] = 0 (x^{-\mu+\frac{1}{2}})$,
Read $D_x^r [\alpha_\mu (x)] = O (x^{-\mu+\frac{1}{2}})$,